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SYNOVIAL FLUID IN RHEUMATOID ARTHRITIS

WITH SPECIAL REFERENCE TO INTRA-ARTICULARLY APPLIED HYDROCORTISONE

BY

PAAVO MÄKINEN

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FROM THE DEPARTMENT OF MEDICAL CHEMISTRY, UNIVERSITY OF TURKU
and FROM THE HOSPITAL OF THE RHEUMATISM FOUNDATION, HEINOLA

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ACADEMIC DISSERTATION

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PREFACE

The present investigation was carried out in the Department of Medical Chemistry, University of Turku, in 1955—1958. The study was suggested and supervised by Professor E. Kulonen, M. D., head of the Department. My deep gratitude is extended to him for his unfailing interest and guidance in all stages of my work. I am also greatly indebted to him for placing the working facilities at the laboratory of the Department at my disposal.

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Paavo Mäkinen

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REVIEW OF LITERATURE

ORIGIN OF SYNOVIAL FLUID

During the past century the view was held that the joint space develops in the embryonic joint anlage as a result of local degeneration of tissue (38, 83). Hagen-Torn (38) and Retterer (82, 83) regarded the embryonic joint fluid to be a mixture of liquefied ground substance and cell decomposition products. Later investigations have shown that the cavitation is the result of the active function of cells (34). According to Vaubel (107) the fluid in the cavity is elaborated by living mesenchymal cells.

A number of theories have been presented concerning the mechanism of formation of the adult synovial fluid. According to the oldest theory, originating with Havers (41), the synovial fluid is a product of »glandular» synovial membrane cells. The secretion theory in a modified form was still recently supported by Kling (55, 56). The theory that the synovial fluid is a transudate from capillaries and lymphatics was presumably first suggested by Bichat (11). Later supporters of the transudation theory considered the synovial fluid mucin a product of disintegration of the synovial membrane (32, 38, 40, 105) or a product of active function of cells in the synovial tissue (60). The theory that the synovial fluid originates in cartilage (66) has received little support.

Hüter (49) was the first to demonstrate that the synovial tissue in fact is connective tissue with no actual membrane and that the joint cavity is an enlarged tissue space. The general view today is that the synovial tissue is a specialized connective tissue and that it does not contain secretory cells (7, 8, 21, 34, 53, 54, 107, 108). Many investigators (19, 49, 53, 54, 85, 107, and others) are of the opinion that the synovial fluid is the liquid matrix

of connective tissue. Corresponding in principle is the theory proposed by Bauer and his co-workers that the synovial fluid is a dialysate of serum, to which mucin is added as the fluid diffuses through the synovial tissue into the joint cavity (90). The presence of protein in the synovial fluid is explained in this theory by a slight capillary permeability to protein.

COMPOSITION AND PROPERTIES OF SYNOVIAL FLUID

The composition and properties of normal synovial fluid have been studied chiefly from human synovial fluid aspirated *post mortem* and the synovial fluid of animals. The first analysis of synovial fluid was carried out by Frerichs (32) in 1846.

The normal synovial fluid contains some amount of cells, albumin, globulin and mucin. Glucose and soluble nitrogenous substances are present approximately in the same concentrations as in the plasma, electrolytes in about the same concentrations as in the plasma dialysates (89). The most definite differences between normal and pathological synovial fluids have been found to exist in the cytological features (18, 22, 43, 89), the protein concentration and the colloidal properties, such as viscosity.

ELECTROLYTES

Ropes, Bennett and Bauer (90) studied the electrolyte concentrations of bovine synovial fluid and serum and observed that the ratio between these concentrations corresponds to the theoretic Donnan equilibrium. In their investigation the sodium and potassium concentrations were lower in synovial fluid than in serum. Yielding, Platt and Holley (110) reported that in persons without joint disease the ratio between synovial fluid sodium and serum sodium was 0.93 in average and the ratio for potassium 1.08, and that in patients suffering from rheumatoid arthritis the ratio for sodium was 0.99 and for potassium 1.03.

Data published by several authors on the electrolyte concentrations of normal and certain pathological synovial fluids are listed in Table 1.

TABLE 1

SODIUM, POTASSIUM AND CHLORIDE CONCENTRATIONS OF NORMAL AND CERTAIN PATHOLOGICAL SYNOVIAL FLUIDS, ACCORDING TO SEVERAL AUTHORS

Authors	Year	Type of Synovial Fluid	Number of Fluids	Characteristic Reported	Sodium, m-equiv./l.	Potassium, m-equiv./l.	Chlorides, m-equiv./l.
Yielding, Platt & Holley (110)	1954	normal, human	10	mean	136.1	4.0	..
— » —	»	normal, post mortem	10	»	133.7	4.8	..
Ropes, Bennett & Bauer (90)..	1939	bovine fluid	36	»	145.0	4.04	110.5
— » —	»	»	»	range	140.1—147.8	3.60—4.40	104.9—116.3
Horiye (47)	1924	edematic	15	»	..	0.6—7.4	..
Fremont-Smith & Dailey (31) ..	1926	traumatic	15	»	..	..	106.0—121.7
Yielding, Platt & Holley (110)	1954	osteoarthritic	5	mean	136.9	4.1	..
— » —	»	rheumatoid	25	»	143.6	4.6	..
Hunziker (48)	1955	»	..	»	151	4.0	102
— » —	»	»	..	range	113—189	3.3—6.8	93—121
Yielding, Platt & Holley (111)	1956	»	34	mean	142.1	4.6	..

PROTEINS

Qualitative studies on proteins in normal synovial fluid carried out by Curtain (20) and Platt, Pigman, Holley and Patton (76) suggested that the proteins, at least for the greater part, are derived from serum. It has not been definitely demonstrated whether or not protein is synthetized in the synovial tissue. Perlmann and Kaufman (73) found in a few cases higher γ-globulin contents in rheumatoid synovial fluid than in the corresponding sera and suggested that this observation pointed to the possible synthesis of protein in the synovial tissue. The removal of protein from the joint cavity occurs, according to Bauer, Short and Bennett (9), entirely by way of the lymphatics.

Findings reported in the literature on the protein concentration of synovial fluid are assembled in Table 2. Risak and Winkler (84) and Keefer, Myers and Holmes (51) observed that in rheumatoid arthritis the total protein concentration of the synovial fluid was higher in joints with severe than in those with milder inflammation. Ropes and Bauer (89) established a correlation between the fluid total protein concentration on the one hand, and the severity and duration of the disease and duration of the effusion, on the other hand. They also observed that in patients with intermittent joint effusion the protein concentration of synovial fluid decreased with increasing effusion and increased with decreasing effusion, and suggested that the entering fluid was lower in protein than the fluid present in the joint cavity. Sundblad (102) observed a positive correlation between the total protein concentration and the volume of fluid in traumatic arthritis and in »arthritis of infectious or unknown etiology» but he did not find this correlation in rheumatoid arthritis.

The albumin/globulin ratio of normal synovial fluid is higher than that of serum (89, 90). Among pathological synovial fluids, according to Ropes and Bauer (89), the traumatic synovial fluid is nearest to normal fluid with regard to the protein ratios. In traumatic synovial fluids they found α_1- and β-globulins in the same relative concentrations as in the corresponding sera, but α_2- and γ-globulins in smaller amounts in the fluids than in the sera. In the series of Schürch, Viollier and Süllmann (98)

TABLE 2

PROTEIN CONCENTRATIONS OF NORMAL AND CERTAIN PATHO-

Authors	Year	Type of Synovial Fluid	Number of Fluids
Sundblad (102)	1953	normal	2
Platt, Pigman Holley & Patton (76)	1956	»	14
Ropes, Rossmeisl & Bauer (92)	1940	normal,	10
— » —	»	post mortem	»
— » —	»	»	1
Ragan (77)	1946	»	6
Platt, Pigman, Holley & Patton (76)	1956	»	11
Horiye (47)	1924	edematic	16
Ropes, Rossmeisl & Bauer (92)	1940	»	60
— » —	»	»	»
— » —	»	»	6
— » —	»	»	»
Ragan (77)	1946	»	5
Allison, Fremont-Smith, Dailey & Kennard (1)	1926	traumatic	13
Collins (19)	1936	»	2
Renkonen (81)	1939	»	31
Schürch, Viollier & Süllmann (98)	1950	»	29
— » —	»	»	»
Kulonen (57)	1951	»	7
Ropes & Bauer (89)	1953	»	30
— » —	»	»	»
— » —	»	»	17
— » —	»	»	»
Sundblad (102)	1953	»	41
Perlmann, Ropes, Kaufman & Bauer (74)	1954	»	6
Cajori, Crouter & Pemberton (15)	1926	rheumatoid	11
Cajori & Pemberton (16)	1928	»	11
— » —	»	»	»
Risak & Winkler (84)	1929	»	5
Fischer (29)	1931	»	10
Keefer, Myers & Holmes (51)	1934	»	9
Collins (19)	1936	»	21
Renkonen (81)	1939	»	7
Ragan (77)	1946	»	5
Ziff, Scull, Ford, McEwen & Bunim (104)	1952	»	4
Jessar, Ganzell & Ragan (50)	1953	»	10
Ropes & Bauer (89)	1953	»	127
— » —	»	»	»
— » —	»	»	61
— » —	»	»	33
Sundblad (102)	1953	»	»
Perlmann, Ropes, Kaufman & Bauer (79)	1954	»	21
Sundblad, Egelius & Jonsson (103)	1954	»	10
Hunziker (48)	1955	»	50
Yielding, Platt & Holley (111)	1956	»	34

LOGICAL SYNOVIAL FLUIDS, ACCORDING TO SEVERAL AUTHORS

Characteristic Reported	Protein Concentrations, g./100 ml.			A/G Ratio
	Total Protein	Albumin	Globulin	
mean	2.8	..	..	..
»	2.8	..	..	2.08
»	1.72	..	..	..
range	1.31—2.12	..	..	..
—	..	1.02	0.05	2.04
range	1.3—2.4	..	..	..
mean	2.0	..	..	1.78
range	0.45—2.49	..	..	..
mean	0.63	..	..	..
range	0.012—1.78	..	..	..
mean	..	0.67	0.17	..
range	..	0.12—1.45	0—0.33	..
»	0.94—2.2	..	..	..
»	3.64—6.33	..	..	..
»	3.25—3.35	..	..	..
»	2.9—6.0	..	..	..
mean	4.35	..	..	..
range	3.68—6.18	..	..	..
»	2—5	..	..	..
mean	3.89	..	..	..
range	2.85—5.45	..	..	..
mean	..	2.97	1.06	..
range	..	2.31—4.04	0.59—2.02	..
mean	4.0	..	..	..
range	2.99—3.90	..	..	..
»	4.18—7.25	..	..	..
mean	4.97	3.00	1.47	2.0
range	3.54—6.02	2.26—3.72	0.31—2.45	1.2—8.8
»	1.0—4.1	..	..	..
»	3.8—7.5	..	..	..
»	2.4—5.1	..	..	..
»	2.72—8.55	..	..	..
»	3.8—6.3	..	..	..
»	4.2—5.9	..	..	..
»	3.4—4.3	1.7—2.6	1.5—1.9	0.9—1.5
»	4.2—6.6	..	..	..
mean	4.77	..	..	..
range	1.74—8.89	..	..	..
mean	..	2.76	2.06	..
range	..	1.91—3.72	0.80—6.77	..
mean	5.1	..	..	..
range	3.98—6.43	..	..	..
mean	4.11	..	..	..
»	4.7	..	..	..
»	5.3	..	..	..

and of Olhagen (72) the electrophoretic protein patterns of traumatic synovial fluid during an incipient stage of the effusion corresponded to those of serum, but in recurrent cases and in cases with persisting effusion of long duration there was a relative increase in the γ-globulin. In some cases of rheumatoid arthritis Ropes and Bauer (89) found even a lower albumin/globulin ratio in the synovial fluid than in the serum. Their observations indicated that the more severe the inflammation of the joint, the greater the percentage increase in globulin. Perlmann and Kaufman (73) and Olhagen (72) found that especially the γ-fraction increased with duration of the disease.

Table 3 gives data reported in the literature on the electrophoretic protein patterns of traumatic and rheumatoid synovial fluids.

HYALURONIC ACID

Ogston and his co-workers (70, 71) wrote that the function of hyaluronic acid in synovial fluid is to contribute to the lubricant and elastic properties of the fluid. Ogston and Stanier (67, 68, 69) concluded that hyaluronic acid exists in synovial fluid in the form of a protein complex, which is a highly hydrated flexible chain polymer. The high intrinsic viscosity of its solutions, in their opinion, is due to the large degree of hydration (68, 69).

The opinion most generally accepted is that the hyaluronic acid of connective tissue is elaborated by fibroblasts (23). Definite knowledge is lacking on the origin of the hyaluronic acid in synovial fluid but the studies carried out by Vaubel (106) and Kulonen (57) with synovial cells *in vitro* have provided evidence that it probably is elaborated by the synovial cells.

The total amount of hyaluronic acid has been found generally to be higher in pathological synovial fluids than in normal fluids, although the concentration may be lower in pathological than in normal fluids. The increase in the total amount has been suggested by Ragan and Meyer (79) and by Kulonen (57) to be a result of an increased production of hyaluronic acid in synovial tissue.

Table 4 gives data on the hyaluronic acid concentration of normal and certain pathological synovial fluids. In rheumatoid

TABLE 3

ELECTROPHORETIC PROTEIN PATTERNS OF TRAUMATIC AND RHEUMATOID SYNOVIAL FLUIDS, ACCORDING TO SEVERAL AUTHORS

Authors	Year	Type of Synovial Fluid	Number of Fluids	Characteristic Reported	Percentage Distribution of Protein Fractions				
					Albumin	Globulins			
						α_1	α_2	β	γ
Schürch, Viollier & Süllmann (98)	1950	traumatic	29	mean	55.3	4.4	7.4	14.1	18.7
— » —	»	»	»	range	47.2—61.6	3.0— 6.8	4.8—13.9	10.4—20.0	11.6—29.9
Perlmann, Ropes, Kaufman & Bauer (74)	1954	»	6	»	61.6—70.3	3.3— 5.5	5.5— 7.7	8.9—14.3	9.4—14.6
— » —	»	rheumatoid	21	»	31.9—57.4	2.9—10.7	4.8—15.5	6.9—18.7	11.3—41.2
Hunziker (48)	1955	»	50	mean	48.6	5.2	9.1	13.2	23.9

TABLE 4

HYALURONIC ACID AND MUCIN CONCENTRATIONS OF NORMAL AND CERTAIN PATHOLOGICAL SYNOVIAL FLUIDS, ACCORDING TO SEVERAL AUTHORS

Authors	Year	Type of Synovial Fluid	Number of Fluids	Characteristic Reported	Hyaluronic Acid, g./100 ml.	Mucin Nitrogen, g./100 ml.	Mucin Glucosamine, g./100 ml.	Mucin Nitrogen/ Mucin Glucosamine Ratio
Sundblad (102)	1953	normal	2	mean	0.297	..	..	..
Ropes, Robertson, Rossmeisl, Peabody & Bauer (91)	1947	normal, post mortem	..	»	..	0.066	..	1.16
Ragan & Meyer (79)	1949	»	13	range	0.04—0.336	..	..	..
Ropes & Bauer (89)	1953	»	5	mean	..	0.104	0.087	..
—»—	»	edematic	48	»	..	0.061	..	..
—»—	»	»	2	»	..	..	0.042	..
Ropes, Robertson, Rossmeisl, Peabody & Bauer (91)	1947	traumatic	..	»	..	0.058	..	0.63
Ropes & Bauer (89)	1953	»	30	»	..	0.068	..	..
—»—	»	»	4	»	..	..	0.078	..
Sundblad (102)	1953	»	55	»	0.150	..	..	..
Levine & Kling (65)	1956	»	8	»	0.148	..	..	..
Ropes, Robertson, Rossmeisl, Peabody & Bauer (91)	1947	rheumatoid	..	»	..	0.067	..	1.30
Ragan & Meyer (79)	1949	»	41	range	0.08—0.287	..	..	..
—»—	»		122	mean	..	0.064	..	..
Jessar, Ganzell & Ragan (50)	1953	»	10	range	0.051—0.140	..	..	..
Ropes & Bauer (89)	1953	»	48	mean	..	..	0.043	..
Sundblad (102)	1953	»	40	»	0.152	..	..	..
Bollet (13)	1956	»	25	range	0.102—0.126	..	..	..
Levine & Kling (65)	1956	»	32	mean	0.158	..	..	..

arthritis Ropes and Bauer (89) observed the lowest concentrations in severe cases of the disease, but Egelius, Jonsson and Sundblad (26) found no correlation between the hyaluronic acid concentration and the activity of the disease or the severity of the joint destructions produced by the disease. Kulonen (57) found a positive correlation between the hyaluronic acid and protein concentrations in pathological synovial fluids. No information is available on sulfur-containing polysaccharides.

VISCOSITY

Figures reported for the viscosity of synovial fluid are assembled in Table 5. Ragan (77), and Ropes and Bauer (89) observed a lower relative viscosity in early rheumatoid arthritis than in advanced cases. In the series of Egelius, Jonsson and Sundblad (26) the values of anomalous viscosity tended to be lower in early cases without bone destructions, and in younger patients.

A positive correlation was obtained by Sundblad (102) between the intrinsic viscosity of the synovial fluid and the blood erythrocyte sedimentation rate in cases of »arthritis of infectious or unknown etiology» but not in rheumatoid arthritis. The intrinsic viscosity was lower in febrile cases of rheumatoid arthritis than in afebrile cases. No correlation was observed by Sundblad (102) between the intrinsic viscosity and the duration of the disease. There was a negative correlation between the intrinsic viscosity and the hyaluronic acid concentration of synovial fluid in his study.

COLLOID OSMOTIC PRESSURE

Ropes, Bennett and Bauer (90) measured the colloid osmotic pressure of 6 bovine synovial fluids and the corresponding sera. The colloid osmotic pressure of the synovial fluid varied in the range of 127—170 mm. H_2O and that of serum in the range of 340—403 mm. H_2O, the means being 150 and 365 mm. H_2O, respectively. By measurement of the freezing point depression, Kulonen (57) determined that the colloid osmotic pressure of the synovial fluid in cases with chronic effusion was slightly higher than that of serum.

TABLE 5

VISCOSITY OF NORMAL AND CERTAIN PATHOLOGICAL

Authors	Year	Type of Synovial Fluid	Number of Fluids
Sundblad (102)	1953	normal	2
Schneider (97)	1925	normal,	31
—»—	»	post mortem	»
Ropes, Rossmeisl & Bauer (92)	1940	»	20
—»—	»	»	»
Ragan (77)	1946	»	9
Ropes, Robertson, Rossmeisl, Peabody & Bauer (91)	1947	»	16
Ragan & Meyer (79)	1949	»	13
Ropes & Bauer (89)	1953	»	22
—»—	»	»	»
Ropes, Rossmeisl & Bauer (92)	1940	edematic	7
—»—	»	»	»
Ragan (77)	1946	»	5
Collins (19)	1936	traumatic	2
Ropes, Robertson, Rossmeisl, Peabody & Bauer (91)	1947	»	..
Ropes & Bauer (89)	1953	»	22
—»—	»	»	»
Sundblad (102)	1953	»	55
—»—	»	»	»
Levine & Kling (65)	1956	»	8
Cajori, Crouter & Pemberton (15)	1926	rheumatoid	9
Cajori & Pemberton (16)	1928	»	11
—»—	»	»	»
Collins (19)	1936	»	37
Ragan (77)	1946	»	5
Ropes, Robertson, Rossmeisl, Peabody & Bauer (91)	1947	»	..
Ragan & Meyer (79)	1949	»	41
—»—	»	»	»
Ziff, Scull, Ford, McEwen & Bunim (109)	1952	»	5
Jessar, Ganzell & Ragan (50)	1953	»	10
Ropes & Bauer (89)	1953	»	79
Sundblad (102)	1953	»	40
—»—	»	»	»
Robinson, Duff & Smith (88)	1954	»	35
—»—	»	»	»
Sundblad, Egelius & Jonsson (103)	1954	»	14
Bollet (13)	1956	»	25
Levine & Kling (65)	1956	»	32
Yielding, Platt & Holley (111)	1956	»	12

On the basis of the results of their measurements and of the theoretic colloid osmotic pressure of protein Ropes, Bennett and Bauer (90) calculated that the colloid osmotic pressure of mucin is nine times as high per gramme as that of serum albumin.

SYNOVIAL FLUIDS, ACCORDING TO SEVERAL AUTHORS

Characteristic Reported	Relative Viscosity	Intrinsic Viscosity	Log. Rel. Visc. / Hyal. Acid. Conc., g./100 ml.	Temperature, °C.
mean	>300	39.3	..	37
»	248.7	..	..	22
range	3.9—1 490	..	..	»
mean	150	..	..	25
range	51—403	..	..	»
»	60—185	..	..	21
mean	203	..	..	38
range	2.3—575.0	..	10.44—20.85	..
mean	235	..	..	38
range	5.7—1 160	..	..	»
mean	53	..	..	25
range	6—174	..	..	»
»	3.0—62.2	..	..	21
»	2.8—11.2	..	..	20
mean	31.1	..	..	38
»	24	..	..	38
range	3.8—83	..	..	»
mean	..	35.6	..	37
range	3—300	..	..	»
mean	..	33.3	..	21.5
range	2.7—16.7	..	..	20
mean	4.08	..	..	20
range	2.91—4.79	..	..	»
»	4.2—32.5	..	..	20
»	5.6—175	..	..	21
mean	13.1	..	..	38
»	123	..	..	..
range	4.5—>1 100	..	4.16—13.80	..
»	3.5—8.1	..	..	38
»	5.7—43.9	..	8.2—17.4	21
mean	16	..	..	38
range	2—34	..	..	37
mean	..	26.3	..	»
»	10.7	..	..	20
range	4.7—47.6	..	..	»
mean	..	27.2	..	37
range	..	5—69	..	37
mean	..	29.1	..	21.5
»	34.3	..	..	37

They suggested it as possible that alterations in the mucin may play a rôle in the mechanism of formation of joint effusion. Kulonen (57), however, could find no effect by the degradation of hyaluronic acid on the freezing point of synovial fluid.

Sundblad (102) observed a negative correlation between the hyaluronic acid concentration and the volume of synovial fluid and a positive correlation between the total protein concentration and the volume of fluid in traumatic arthritis, but did not find these correlations in rheumatoid arthritis. There was no correlation in either group between the intrinsic viscosity and the fluid volume.

EFFECTS OF CORTICOSTEROIDS ON CONNECTIVE TISSUE AND SYNOVIAL FLUID

Clinical observations and numerous experimental studies have shown that several hormones regulate the biological events in connective tissue and that disturbances in the endocrine system induce pathological changes in this tissue. It is known that at least somatotrophin, thyrotrophin, thyroxine, adrenocortical hormones, sex hormones (3, 4) and insulin (95) directly participate in the regulatory mechanism of connective tissue.

The general observation made on the effects of corticosteroids on connective tissue is that they have an inhibitory action on the connective tissue functions. It is held that their significance in inflammatory conditions is based on their ability to check inflammatory processes. In Table 6 are listed some of the effects that corticosteroids have been found to have on connective tissue. Only a few of the first papers published on each observation are mentioned in this table.

The favorable effect of local treatment with cortisone on certain inflammatory conditions of the joints was first observed by Thorn (104) and Hollander (44) in 1950 and with hydrocortisone by Hollander, Brown, Jessar and Brown (45) in 1951. A large number of reports on the clinical effects of intra-articular hydrocortisone treatment have been published subsequently. The abatement of inflammatory symptoms from the joint and decrease in the joint effusion have been general perceptions. Observations made concerning the effects of corticosteroid hormones on the composition and properties of the synovial fluid are listed in Table 7.

TABLE 6

OBSERVATIONS REPORTED IN THE LITERATURE ON THE EFFECTS OF CORTICOSTEROIDS ON CONNECTIVE TISSUE

Authors	Year	Observations
		Effects on connective tissue in general
Ragan, Howes, Plotz, Meyer & Blunt (78)	1949	Delay in the development of all elements of connective tissue, in division and proliferation of cells and formation of ground substance and fibers
Seifter, Baeder & Begany (99)	1949	Decrease in permeability of connective tissue
Asboe-Hansen (2)	1950	Decrease in number of mast cells, reduction of ground substance
Castor & Baker (17)	1950	Diminishing in number and size of fibroblasts, homogenization of collagen fiber structure
Green (37)	1950	Inhibitory action on mitoses
Layton (63)	1951	Reduction of synthesis of sulphonated polysaccharides
Roberts, Karnofsky & Frankel (87)	1951	Reduction of formation of collagen
Lattes, Blunt, Rose, Jessar, de Guise Vaillancourt & Ragan (62)	1953	Decrease in metachromasy of connective tissue
Schiller & Dorfman (94)	1957	Reduction of synthesis of hyaluronic acid
		Effects on synovial tissue
Giansiracusa, Ropes, Kulka & Bauer (35)	1950	Reduction of histological signs of inflammation in synovial tissue
Hench, Kendall, Slocumb & Polley (42)	1950	Reduction of histological signs of inflammation in synovial tissue
Reed, Joseph, Steck & Montgomery (80)	1950	Lowering in potential difference between synovial membrane and skin
Hollander, Brown, Jessar & Brown (45)	1951	Decline in intra-articular temperature in inflamed joints
Kulonen & Telkkä (59)	1954	Inhibitory action on synovial cells
Edlund, Juhlin & Palis (25)	1955	Decrease in permeability of synovial membrane

TABLE 7

OBSERVATIONS REPORTED IN THE LITERATURE ON THE EFFECTS OF COSTICOSTEROIDS ON PATHOLOGICAL SYNOVIAL FLUIDS

Authors	Year	Observations
Duff, Robinson & Smith (24)	1951	Decrease in cell count, increase in degree of polymerization of hyaluronic acid
Kulonen (57)	1951	Rise in relative viscosity
Ziff, Scull, Ford, McEwen & Bunim (109)	1952	Fall in cell content and aminotripeptidase content, rise in relative viscosity, no definite effect on total protein and albumin/globulin ratio
Jessar, Ganzell & Ragan (50)	1953	Increase in hyaluronic acid concentration, in relative viscosity and in degree of polymerization of hyaluronic acid, no definite effect on total protein
Ropes & Bauer (89)	1953	Decrease in cell count, increase in relative viscosity
Ekman, Thune & Truedsson(27)	1953	Increase in hyaluronidase inhibitor activity
Sundblad, Egelius & Jonsson (103)	1954	Increase in hyaluronic acid concentration and in degree of polymerization of hyaluronic acid, no effect on total protein and hyaluronidase inhibitor activity
Kulonen & Mäkinen (58)	1955	Decrease in water-binding capacity and in reactive amino groups
Robecchi, Daneo & Riccardino (86)	1955	Decrease in total protein concentration, relative increase in albumin fraction, no effect on γ-globulin
Borrachero (14)	1956	Increase in albumin concentration, decrease in β- and γ-globulin concentration, no effect on α-globulin
Yielding, Platt & Holley (111)	1956	Decline in sodium concentration, increase in relative viscosity, no effect on potassium and total protein concentrations
Platt, Holley & Pigman (75)	1957	Partial normalization in the relative electrophoretic mobilities of albumin, α_1-, α_2- and β-globulins, no definite effect on γ-globulin

PRESENT INVESTIGATION

PURPOSE OF INVESTIGATION

The following problems formed the point of departure for the present study:

1. Do the chemical and physico-chemical properties of synovial fluid have any relationship to the clinical features of rheumatoid arthritis?

2. What kind of changes will be induced by intra-articular corticosteroid treatment in the chemical and physico-chemical properties of the synovial fluid in rheumatoid arthritis?

3. What conclusions concerning the mechanism of formation of joint effusion in rheumatoid arthritis can be drawn from the results?

MATERIAL AND METHODS

The chemical and physico-chemical examination of the synovial fluid in rheumatoid arthritis was made from samples aspirated from the affected knee joint of patients before and after an intra-articular injection of hydrocortisone acetate. For comparison, thyroxine or insulin was substituted for the hydrocortisone in a few cases. Sodium, potassium, chloride, total protein, protein glucosamine and hyaluronic acid concentrations, electrophoretic protein patterns, intrinsic viscosity, and colloid osmotic pressure were examined from the samples.

A total of 63 pairs of samples were collected from 63 knee joints of 56 patients with rheumatoid arthritis. [1] The series included only cases in which the diagnosis of rheumatoid arthritis was definitely verified with clinical and roentgenological examinations and which has not been treated with any hormones for at least the preceding six weeks before the sampling procedure. Complete clinical and roentgenological examinations of the most markedly affected joints and the knee joints were made, and the stage of progression and grade of clinical activity of the disease, the grade of articular changes and degree of effusion in the knee joints under examination, and the clinical effect of the intra-articular hydrocortisone treatment were recorded. For the treatment of the results the series was distributed according to the clinical and roentgenological criteria stated below, and attempts were made to find out, by means of statistical methods, (1) what properties of synovial fluid could be shown to have relationship with the clinical characteristics of the disease and (2) what changes in the properties of the fluid occur through the action of intra-articularly injected hydrocortisone.

[1] The series includes also the cases presented in a paper by Kulonen and Mäkinen in 1955 (58).

CLINICAL CRITERIA

The progress of the disease was defined by the system of Steinbrocker, Traeger and Batterman (101). The material was divided into two groups by means of the roentgenological findings in the most severily affected joint, one group including the cases with no joint destruction (stages I and II) and the other group the cases in which joint destructions or ankylosis were found (stages III and IV).

The clinical activity of the disease was determined by the system of Bayles and Hall (10). The values (0—4) given under this system to the joint changes (synovial thickening, joint stiffness, osseal deformity, ankylosis) in the most markedly affected joint, the inflammatory symptoms (pain, swelling, heat, redness) in the most severily inflamed joint, elevation of the blood erythrocyte sedimentation rate, decline of the blood hemoglobin content, and decrease in the patient's functional capacity were added up. The cases in which the sum was 4—11 were collected into one group (»mild or moderate») and those with a sum of 12—18 were placed in another group (»severe»).

The grade of local articular changes was estimated by the system of Kellgren and Lawrence (52). In addition to the osteoporosis and erosions in the articulating bones, the changes involving cartilage were also indicated by values 0—4. The distribution of the cases was based on the sum of these three values. The joint changes were regarded as »mild or moderate» if the sum was 1—5, and »severe» if the values totaled 6—10.

The degree of joint effusion was estimated from the circumference of the joint, the volume of fluid obtained by the second puncture, and the findings at palpation. The effusion was designated as »moderate», »large» or »very large» in degree. No direct measurement or indirect determination of the volume of effusion fluid before the injection of hydrocortisone was performed, in order not to cause synovial irritation or disturb the biochemical examinations. Fluid samples obtained from small effusions were inadequate for the examinations and were discarded.

The clinical effect of the intra-articular hydrocortisone treatment was estimated using as criteria diminished hydrops, increased joint mobility, and abatement of pain. The result of the treatment was designated as »poor», »moderate» or »good».

FLUID SAMPLES

The samples of synovial fluid were obtained from the knee joint by puncture, using a No. V2A hypodermic needle. After 10 ml. of fluid had been drawn into the syringe, 25—50 mg. of hydrocortisone acetate (Hydrocortone Acetate Suspension, Merck) was injected into the joint cavity during the same puncture. Instead of hydrocortisone, 1 ml. of thyroid extract (Thyranon, Organon) was injected in 4 cases and 20 IU of regular insulin (Insulin, Medica) in 4 cases. A second puncture was performed 24 hours later and another sample was withdrawn. All the remaining fluid was removed at the second puncture and the volume was measured. The samples were placed in dry, sterilized vials and stored at first in the refrigerator at —7 °C, later in the deep-freezer at —15 °C. Before examination the samples were defrozen and centrifuged clear. Some of the collections were scarcely colored by blood. None of the fluid samples contained pus.

CHEMICAL AND PHYSICO-CHEMICAL METHODS

The *sodium* and *potassium* concentrations were determined with a flame photometer using an internal lithium standard according to Leppänen, Krusius and Miettinen (64). The sodium determinations were made from fluid diluted 1 : 500 and the potassium determinations from a dilution 1 : 50. The *chloride* content was determined by the mercurimetric method according to Schales and Schales (93). The corresponding values for normal plasma by these methods are in average 140—145 m-equiv./l. of sodium, 4.3 m-equiv./l. of potassium, and 105 m-equiv./l. of chlorides.

Determination of the *total protein* concentration was carried out by the biuret method from 0.16 ml. of synovial fluid treated with hyaluronidase to facilitate pipetting. The hyaluronidase treatment was performed as follows: A hyaluronidase solution was prepared by dissolving 10 mg. (1300 TRU) of testicular hyaluronidase (originally prepared by Dr. M. B. Mathews) per ml. of 0.5 *M* acetate buffer, pH 4.5. Then 0.25 ml. of hyaluronidase solution was added to 1 ml. of synovial fluid and the mixture

was kept for 40 hours at + 37° C., using a few drops of toluene as preservative.

The *fractionation of proteins* was performed by paper electrophoresis using Whatman No. 1 paper and a voltage gradient of about 10 V/cm. Before the electrophoresis the synovial fluid was treated with hyaluronidase and buffered with 0.05 *M* barbiturate buffer to pH 8.6. The strips were stained with Amidoblack 10 B and washed with methanol according to Grassmann and Hannig (36). The fractions were cut, eluted with alkaline methanol and the color was measured.

The *glucosamine* concentration was determined by the principle of Blix (12). The standard was made from glucosamine hydrochloride (B. D. H.). To obtain the *hyaluronic acid* concentration the difference between the total glucosamine and protein glucosamine concentrations was multiplied by the factor 2.36.

The *protein glucosamine* concentration was determined as follows: 1 ml. of synovial fluid was treated with hyaluronidase in the manner described above. The protein was precipitated from the mixture with 4 ml. of 95 per cent ethanol. The precipitate was washed with 10 per cent trichloroacetic acid solution, dried and hydrolyzed for the glucosamine determination.

The *intrinsic viscosity* was determined with the Ubbelohde viscosimeter, using dilutions 1 : 20—1 : 80. The determinations were made from at least three dilutions of each sample. The diluent was a 0.9 per cent sodium chloride solution, buffered with 1/150 *M* phosphate buffer to pH 6.8. Two viscosimeters were used, each with a bulb capacity of about 2.5 ml. and a capillary diameter of 0.5 mm. The flow time of the diluent was 100 seconds in one and 120 seconds in the other case. The temperature in the water bath was controlled at + 32° C. with an accuracy of 0.1° C. The intrisic viscosity was calculated according to the following formula given by Sundblad (102):

$$\text{intrinsic viscosity } [\eta] = \underset{\text{lim. } c \to 0}{\frac{\eta_{sp.}}{c}} = \frac{\eta_{sp.}}{c} \Big/ (1 + 0.18 \times \eta_{sp.})$$

The *colloid osmotic pressure* was measured by the method of Autio (6). The principle of the method is as follows: A Holm-

Jensen osmometer is filled with a reference solution and is placed in the vertical position with the membrane upward. The capillary tube of the osmometer is connected to a hydrostatic system with which a negative pressure can be produced in the osmometer. The fluid to be examined is placed on the membrane. The negative pressure necessary to prevent all movements of the fluid across the membrane was recorded. Saline solution (0.9 %) was used for the comparative fluid. The same membrane, prepared and tested according to the instructions of Holm-Jensen (5, 46), was used for all the measurements.

STATISTICAL METHODS

Standard methods (28, 30) were used for the statistical analyses of the results. The relations of the biochemical observations to the clinical activity of the disease, the stage of the disease, and the grade of local joint changes were studied by calculating the group means and applying the *t*-test to the difference of the means. A comparison with the degree of joint effusion was made by studying the significance of the differences of the group means by variance analysis. The analyses of the effects of the hormone treatments on the properties of the synovial fluid were studied by calculating the changes in the individual pairs and applying the *t*-test to the mean changes. The correlations between the blood erythrocyte sedimentation rate and the observed values of biochemical characteristics of the synovial fluid, and the mutual correlations of the various biochemical observations were studied by means of regression and variance analysis on the cases in which a preliminary graphic analysis prompted a mathematical treatment. The differences of the means and the correlations were regarded as statistically significant when the value of the probability of the chance occurrence (P) was not more than 0.05, and as highly significant when the value of P was at the most 0.001. In the tables and figures $P = 0.05$ is indicated by *, $P = 0.01$ by ** and $P = 0.001$ by ***. Hald's (39) tables were used in the statistical analyses.

RESULTS

CLINICAL DATA

In Table 8 clinical data are presented on the series of patients and on the distribution of the series according to the clinical findings.

TABLE 8

MATERIAL

Clinical Argument	Number	Mean	Range
Patients	56	—	—
Applied joints	63	—	—
General features of the disease			
Age of patients, years	—	36	14—58
Duration of disease, years	—	7	1/2—26
Stage of disease: I and II	35	—	—
Stage of disease: III and IV	21	—	—
Clinical activity of disease: mild or moderate	28	—	—
Clinical activity of disease: severe	28	—	—
Blood erythrocyte sedimentation rate, mm./1 hr.	—	51	9—130
Local articular changes: mild or moderate	28	—	—
Local articular changes: severe	35	—	—
Degree of effusion: moderate	24	—	—
Degree of effusion: large	25	—	—
Degree of effusion: very large	14	—	—
Intra-articular hydrocortisone treatment	55	—	—
Clinical effect: poor	12	—	—
Clinical effect: moderate	13	—	—
Clinical effect: good	30	—	—
Intra-articular thyroxine treatment	4	—	—
Intra-articular insulin treatment	4	—	—

CHEMICAL AND PHYSICO-CHEMICAL PROPERTIES OF RHEUMATOID SYNOVIAL FLUID

Table 9 shows the means and ranges of the values obtained in the chemical and physico-chemical examinations of the synovial fluid.

TABLE 9

BIOCHEMICAL FINDINGS IN RHEUMATOID SYNOVIAL FLUID

Investigation	Number of Fluids	Mean	Range
Electrolytes, m-equiv./1.			
Sodium	63	148.6	120—188
Potassium	62	4.29	3.18—6.28
Chlorides	63	107.4	87—138
Proteins, g./100 ml.			
Total protein	57	5.84	4.0 —7.5
Albumin	11	3.28	1.61—4.65
α-globulin	6	0.58	0.46—0.67
β-globulin	11	0.73	0.37—1.29
γ-globulin	11	1.58	1.03—2.32
Protein glucosamine, mg./100 ml.	58	58.9	22—91
Hyaluronic acid, g./100 ml.	58	0.233	0.13—0.44
Intrinsic viscosity	58	14.0	6.5—22.4
Colloid osmotic pressure, cm. H_2O	32	42.4	34—51

CLINICAL CORRELATIONS OF DIFFERENT PROPERTIES OF SYNOVIAL FLUID

Electrolytes. — The sodium, potassium and chloride concentrations of the synovial fluid had no relationship with the stage of the disease, the grade of local joint changes, or the degree of effusion. The sodium and chloride concentrations showed no relation even to the clinical activity of the disease. The potassium concentration of the fluid was higher in the group of clinically very active cases ($P = 0.01$), and a positive correla-

TABLE 10

POTASSIUM CONCENTRATION OF SYNOVIAL FLUID, BY CLINICAL ACTIVITY OF THE DISEASE

Characteristic	Activity of the Disease	
	Mild or Moderate	Severe
Number of fluids	33	29
Potassium, m-equiv./1.		
— mean	4.02	4.59**
— range	3.18—5.82	3.50—6.28
— standard deviation	0.68	0.73
— standard error of mean	0.118	0.136

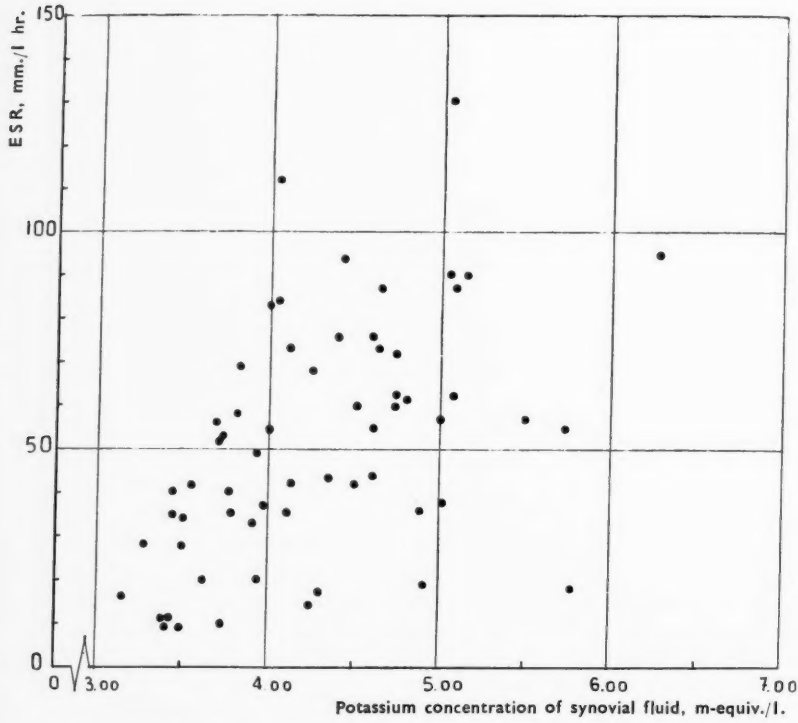

Fig. 1. — Diagram showing the correlation between the potassium concentration of synovial fluid and the blood erythrocyte sedimentation rate ($r = + 0.41$ **)

tion was observed between the potassium concentration and the blood erythrocyte sedimentation rate ($P = 0.002$). The potassium concentration of the fluid in groups formed according to the clinical activity of the disease is shown in Table 10, and the correlation between the potassium concentration of the fluid and the blood erythrocyte sedimentation rate in Fig. 1.

Total Protein. — No relationship was observed between the total protein concentration of the synovial fluid and the clinical activity of the disease. A suggestive but statistically not significant difference between the groups representing different stages of the disease was seen in the total protein concentration (stages I and II: 6.01 g./100 ml., stages III and IV: 5.55 g./100 ml.). A comparison of the total protein concentration in the different effusion groups revealed suggestive but statistically not signifi-

TABLE 11

TOTAL PROTEIN CONCENTRATION OF SYNOVIAL FLUID, BY DEGREE OF THE JOINT EFFUSION (STAGES I AND II)

Characteristic	Degree of the Effusion		
	Moderate	Large	Very Large
Number of fluids	9	16	11
Total protein, g./100 ml.			
— mean	5.71	5.77	6.59**
— range	5.3—7.0	5.1—6.7	4.4—7.2
— standard deviation	0.61	0.58	0.85
— standard error of mean	0.203	0.145	0.256

cant differences between the groups (moderate: 5.59 g./100 ml., large: 5.79 g./100 ml., very large: 6.25 g./100 ml.) in the entire series. When the cases in stages I and II only were included (Table 11) the differences between the mean concentrations were significant ($P = 0.01$).

Protein Glucosamine. — No difference between the disease stage groups was found in the protein glucosamine concentration. The concentration was lower in fluids from joints presenting marked changes than in fluids from joints with slight or moderate changes ($P = 0.001$). A higher concentration was observed in cases with a high clinical activity than in those with a low or moderate activity ($P = 0.02$). Correspondingly, there was a positive correlation between the protein glucosamine concentration and the blood erythrocyte sedimentation rate ($P=0.001$).

TABLE 12

PROTEIN GLUCOSAMINE CONCENTRATION OF SYNOVIAL FLUID, BY CLINICAL FEATURES OF THE DISEASE

Characteristic	Activity of Disease		Stage of Disease		Local Articular Changes	
	Mild or Moderate	Severe	I and II	III and IV	Mild or Moderate	Severe
Number of fluids	29	29	37	21	32	26
Protein glucosamine, mg./100 ml						
— mean	54.0	63.9*	59.0	58.9	65.1	51.7***
— range	39—78	22—91	22—91	39—85	39—91	22—70
— standard deviation	13.4	16.2	16.8	13.0	15.3	12.2
— standard error of mean	2.48	3.00	2.76	2.84	2.70	2.40

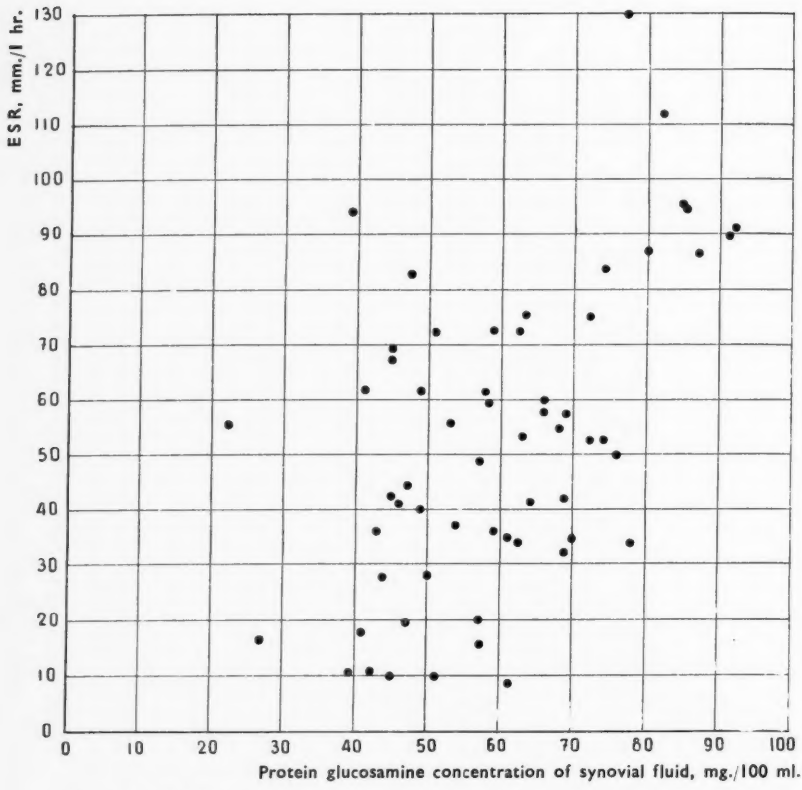

Fig. 2. — Diagram showing the correlation between the protein glucosamine concentration of synovial fluid and the blood erythrocyte sedimentation rate ($r = + 0.52$ ***)

Table 12 shows the relationship of the protein glucosamine concentration of the fluid with the clinical findings, and Fig. 2 the correlation between this concentration and the blood erythrocyte sedimentation rate. The protein glucosamine concentration was not found to be related to the degree of effusion.

Hyaluronic Acid. — The hyaluronic acid concentration of the synovial fluid was not found to have any relationship with the clinical activity or stage of the disease, the grade of local joint changes, or the degree of the effusion.

Intrinsic Viscosity. — In the group of cases in stages I and II the intrisic viscosity was lower than in the group of cases in stages III and IV ($P = 0.02$). The mean value was lower also

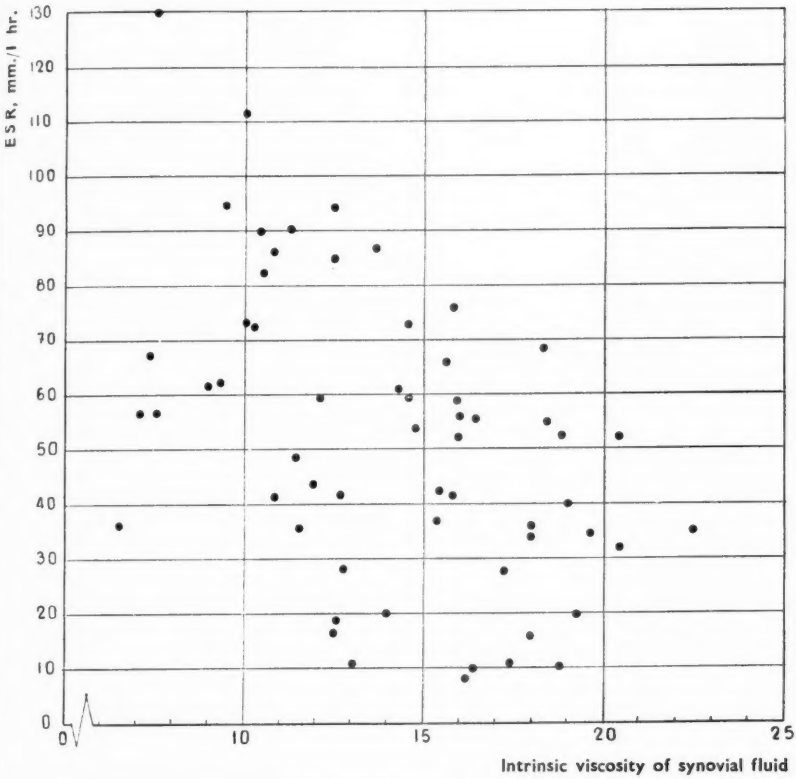

Fig. 3. — Diagram showing the correlation between the intrinsic viscosity of synovial fluid and the blood erythrocyte sedimentation rate ($r = -0.47$ ***)

in the group with slight or moderate local joint changes than in the group of cases with marked local changes, but this difference of the means was statistically not significant. A comparison with the activity of the disease revealed that the intrinsic viscosity was lower in cases with a high clinical activity than in cases with a low or moderate activity ($P = 0.001$). The intrinsic viscosity also showed a negative correlation to the blood erythrocyte sedimentation rate ($P = 0.001$). No relationship was observed between the intrinsic viscosity and the degree of the effusion. Table 13 shows the relations of the intrinsic viscosity to the clinical findings and Fig. 3 the correlation to the blood erythrocyte sedimentation rate.

TABLE 13

INTRINSIC VISCOSITY OF SYNOVIAL FLUID, BY CLINICAL FEATURES OF THE DISEASE

Characteristic	Activity of Disease		Stage of Disease		Local Articular Changes	
	Mild or Moderate	Severe	I and II	III and IV	Mild or Moderate	Severe
Number of fluids	29	29	37	21	32	26
Intrinsic viscosity						
— mean	15.7	12.3***	13.1	15.5*	13.3	14.8
— range	9.3—22.4	6.5—18.4	6.5—22.4	9.3—20.4	6.5—20.4	7.1—22.4
— standard deviation	3.53	3.42	3.82	3.42	4.0	3.6
— standard error of mean	0.65	0.63	0.63	0.75	0.71	0.71

Colloid Osmotic Pressure. — Table 14 gives a comparison of the colloid osmotic pressure of the synovial fluid with the clinical activity and stage of the disease and the local joint changes. The colloid osmotic pressure was higher in cases in stages I and II than in those of stages III and IV ($P = 0.01$). The differences between the means of the groups formed according to the activity of the disease and according to the local joint changes were statistically not significant. A positive correlation ($P = 0.001$) was observed between the colloid osmotic pressure of the fluid and the blood erythrocyte sedimentation rate (Fig. 4). The colloid osmotic pressure was highest in the group of cases with a very large effusion, and lowest in the group with a moderate

TABLE 14

COLLOID OSMOTIC PRESSURE OF SYNOVIAL FLUID, BY CLINICAL FEATURES OF THE DISEASE

Characteristic	Activity of Disease		Stage of Disease		Local Articular Changes	
	Mild or Moderate	Severe	I and II	III and IV	Mild or Moderate	Severe
Number of fluids	14	18	21	11	17	15
Colloid osmotic pressure, cm. H_2O						
— mean	41.1	43.4	44.1	39.1***	43.1	41.5
— range	34—48	35—51	38—51	34—43	35—50	34—51
— standard deviation	4.1	4.1	3.7	3.0	4.0	4.4
— standard error of mean	1.09	0.97	0.81	0.90	0.97	1.14

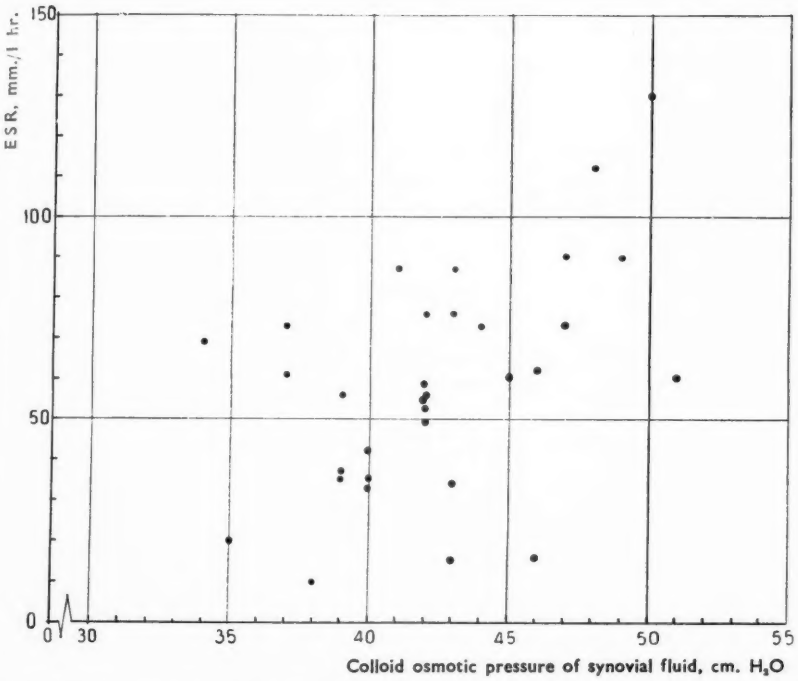

Fig. 4. — Diagram showing the correlation between the colloid osmotic pressure of synovial fluid and the blood erythrocyte sedimentation rate ($r = + 0.70$ ***)

effusion. The difference of the means was statistically significant ($P = 0.05$) when the cases in stages I and II only were included (Table 15) but not significant in the entire series (moderate: 40.9 cm. H_2O, large: 42.7 cm. H_2O, very large: 44.0 cm. H_2O).

TABLE 15

COLLOID OSMOTIC PRESSURE OF SYNOVIAL FLUID, BY DEGREE OF THE JOINT EFFUSION (STAGES I AND II)

Characteristic	Degree of Effusion		
	Moderate	Large	Very Large
Number of fluids	7	8	6
Colloid osmotic pressure, cm. H_2O			
— mean	41.8	44.3	46.3*
— range	38—46	40—50	42—51
— standard deviation	3.3	3.6	3.5
— standard error of mean	1.25	1.27	1.43

INFLUENCE OF INTRA-ARTICULARLY APPLIED HYDROCORTISONE ON SYNOVIAL FLUID

Electrolytes. — The influence of the various hormone treatments on the sodium, potassium and chloride concentrations of synovial fluid will be seen from Tables 16—18. The changes in the sodium and chloride concentrations were statistically not

TABLE 16

INFLUENCE OF INTRA-ARTICULAR HYDROCORTISONE TREATMENT ON SODIUM CONCENTRATION OF SYNOVIAL FLUID

Group and Characteristic	Number of Sample Pairs	Sodium, m-equiv./l.	
		Before Treatment	After Treatment
Hydrocortisone treatment			
poor clinical response	11		
— mean		149.3	151.0
— range		142—164	138—172
— standard deviation		7.2	12.2
— standard error of mean		2.08	3.68
moderate clinical response	13		
— mean		151.5	150.0
— range		120—183	128—173
— standard deviation		19.8	13.2
— standard error of mean		5.49	3.66
good clinical response	30		
— mean		148.0	145.1
— range		123—188	127—165
— standard deviation		10.5	8.1
— standard error of mean		1.88	1.48
total hydrocortisone group	54		
— mean		149.1	147.5
— range		120—188	127—173
— standard deviation		12.6	10.7
— standard error of mean		1.70	1.46
Comparative groups			
thyroxine treatment	4		
— mean		145.0	142.7
— range		141—148	142—144
— standard deviation		3.5	0.9
— standard error of mean		1.75	0.45
insulin treatment	4		
— mean		145.5	135.7
— range		143—149	108—148
— standard deviation		2.9	17.6
— standard error of mean		1.45	8.80

significant either in the hydrocortisone group or in the comparative groups. The decrease in the potassium concentration was significant in the group in which a good clinical response was obtained to the hydrocortisone treatment ($P = 0.01$) and in the hydrocortisone group as a whole ($P = 0.01$). The decline in the potassium level in the thyroxine-group was not significant.

TABLE 17

INFLUENCE OF INTRA-ARTICULAR HYDROCORTISONE TREATMENT ON POTASSIUM CONCENTRATION OF SYNOVIAL FLUID

Group and Characteristic	Number of Sample Pairs	Potassium, m-equiv./l.	
		Before Treatment	After Treatment
Hydrocortisone treatment			
poor clinical response	12		
— mean		4.24	4.09
— range		3.42—5.50	3.05—6.05
— standard deviation		0.71	0.90
— standard error of mean		0.20	0.26
moderate clinical response	13		
— mean		4.02	3.75
— range		3.50—4.65	3.02—4.77
— standard deviation		0.38	0.58
— standard error of mean		0.11	0.16
good clinical response	29		
— mean		4.31	3.79**
— range		3.18—6.28	2.70—6.03
— standard deviation		0.81	0.69
— standard error of mean		0.15	0.13
total hydrocortisone group	54		
— mean		4.23	3.85**
— range		3.18—6.28	2.70—6.05
— standard deviation		0.69	0.71
— standard error of mean		0.09	0.09
Comparative groups			
thyroxine treatment	4		
— mean		4.75	4.21
— range		3.95—5.73	3.43—5.05
— standard deviation		0.84	0.83
— standard error of mean		0.42	0.42
insulin treatment	4		
— mean		4.62	4.60
— range		4.00—5.08	3.92—5.75
— standard deviation		0.55	0.81
— standard error of mean		0.28	0.41

TABLE 18

INFLUENCE OF INTRA-ARTICULAR HYDROCORTISONE TREATMENT ON CHLORIDE CONCENTRATION OF SYNOVIAL FLUID

Group and Characteristic	Number of Sample Pairs	Chlorides, m-equiv./l.	
		Before Treatment	After Treatment
Hydrocortisone treatment			
poor clinical response	12		
— mean		108.2	109.5
— range		103—115	99—119
— standard deviation		3.6	5.8
— standard error of mean		1.04	1.67
moderate clinical response	13		
— mean		107.3	108.0
— range		87—138	100—112
— standard deviation		11.4	4.1
— standard error of mean		3.16	1.18
good clinical response	30		
— mean		106.5	106.7
— range		91—122	93—116
— standard deviation		7.1	6.5
— standard error of mean		1.30	1.19
total hydrocortisone group	55		
— mean		107.1	107.6
— range		87—138	93—119
— standard deviation		8.0	5.7
— standard error of mean		1.08	0.77
Comparative groups			
thyroxine treatment	4		
— mean		110.7	107.0
— range		107—114	105—109
— standard deviation		2.9	1.9
— standard error of mean		1.45	0.95
insulin treatment	4		
— mean		109.2	104.5
— range		107—112	90—114
— standard deviation		1.8	10.1
— standard error of mean		0.90	5.05

Proteins. — The mean concentrations of total protein before and after treatment are shown in Table 19. The decrease in protein after hydrocortisone treatment was statistically significant in the group with a good clinical response ($P = 0.01$) and in all the cases treated with hydrocortisone ($P = 0.02$). The decrease in the groups with a poor or moderate clinical response

TABLE 19

INFLUENCE OF INTRA-ARTICULAR HYDROCORTISONE TREATMENT ON TOTAL PROTEIN CONCENTRATION OF SYNOVIAL FLUID

Group and Characteristic	Number of Sample Pairs	Total Protein, g./100 ml.	
		Before Treatment	After Treatment
Hydrocortisone treatment			
poor clinical response	11		
— mean		5.72	5.49
— range		4.2—7.5	3.2—7.5
— standard deviation		1.13	1.22
— standard error of mean		0.34	0.37
moderate clinical response	13		
— mean		5.65	5.42
— range		4.0—7.2	3.4—7.7
— standard deviation		0.80	1.16
— standard error of mean		0.22	0.32
good clinical response	25		
— mean		6.11	5.59**
— range		4.5—7.2	3.0—7.6
— standard deviation		0.80	1.21
— standard error of mean		0.16	0.24
total hydrocortisone group	49		
— mean		5.88	5.52*
— range		4.0—7.5	3.0—7.7
— standard deviation		0.89	1.10
— standard error of mean		0.13	0.17
Comparative groups			
thyroxine treatment	4		
— mean		5.40	5.55
— range		4.5—6.3	4.5—6.7
— standard deviation		0.89	1.00
— standard error of mean		0.44	0.50
insulin treatment	4		
— mean		5.82	6.20
— range		5.7—6.0	6.0—6.3
— standard deviation		0.13	0.14
— standard error of mean		0.07	0.07

and the increase in the comparative groups were statistically not significant.

In Table 20 are presented the absolute albumin and globulin concentrations before and after the intra-articular application of hydrocortisone. In this series of samples the decrease in the total protein was statistically not significant. The level declined

TABLE 20

INFLUENCE OF INTRA-ARTICULAR HYDROCORTISONE TREATMENT ON ELECTROFORETIC PROTEIN PATTERNS OF SYNOVIAL FLUID

Clinical Response	Total protein, g./100 ml.		Albumin, g./100 ml.		Globulins, g./100 ml.					
					α		β		γ	
	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
Poor	4.1	4.1	1.61	1.42	0.46	0.46	0.54	0.44	1.49	1.78
Poor	4.2	3.2	2.24	1.54	0.51	0.41	0.37	0.34	1.08	0.91
Poor	7.2	5.5	4.12	3.09	..	..	0.76	0.54	2.32	1.87
Poor	7.5	6.2	4.60	3.83	..	..	1.03	0.64	1.87	1.73
Moderate	5.5	6.7	2.97	3.74	..	..	0.62	0.84	1.91	2.12
Moderate	6.3	4.3	3.12	1.85	0.66	0.49	1.29	0.76	1.22	1.20
Good	4.6	5.1	2.36	2.59	0.52	0.63	0.69	0.49	1.03	1.39
Good	5.3	4.9	2.78	2.27	0.67	0.71	0.50	0.48	1.35	1.44
Good	6.0	6.2	3.02	3.21	0.65	0.67	0.73	0.60	1.60	1.72
Good	7.1	7.4	4.59	4.71	..	..	0.72	0.70	1.79	1.99
Good	7.2	7.2	4.65	4.72	..	..	0.80	0.71	1.75	1.77
Mean	5.91	5.53	3.28	2.99	(0.58)	(0.56)	0.73	0.59*	1.58	1.63

in 5 cases, increased in 4 cases and was unchanged in 2 cases. In the cases in which the total protein level was decreased or increased, a change in the same direction occurred in the albumin concentration and with one exception in the α-globulin concentration. The change in mean values was statistically not significant even in these respects. The β-globulin concentration was decreased in all cases but one. The change in the mean value was statistically significant ($P = 0.05$). The increase in the γ-globulin concentration was statistically not significant when all the cases were included, but it was significant ($P = 0.05$) in the group of those cases in which the clinical response was good. The mean γ-globulin concentration in these 5 cases was 1.50 g./100 ml. before the hydrocortisone treatment and 1.66 g./100 ml. after the treatment.

Protein Glucosamine. — Table 21 shows the protein glucosamine concentrations before and after hormone treatments. The decrease was statistically significant in the group in which a good clinical response to hydrocortisone was obtained ($P = 0.01$) and in the hydrocortisone group as a whole ($P = 0.01$) but not significant in the other groups.

TABLE 21

INFLUENCE OF INTRA-ARTICULAR HYDROCORTISONE TREATMENT ON PROTEIN GLUCOSAMINE CONCENTRATION OF SYNOVIAL FLUID

Group and Characteristic	Number of Sample Pairs	Protein Glucosamine, mg./100 ml.	
		Before Treatment	After Treatment
Hydrocortisone treatment			
poor clinical response	11		
— mean		57.5	50.4
— range		22—91	29—70
— standard deviation		19.1	13.2
— standard error of mean		5.75	3.98
moderate clinical response	13		
— mean		58.0	56.5
— range		44—82	39—89
— standard deviation		13.7	14.2
— standard error of mean		3.80	3.94
good clinical response	26		
— mean		58.0	49.4**
— range		27—87	23—84
— standard deviation		15.0	13.6
— standard error of mean		2.94	2.67
total hydrocortisone group	50		
— mean		57.9	51.4**
— range		22—91	23—89
— standard deviation		15.4	13.8
— standard error of mean		2.18	1.95
Comparative groups			
thyroxine treatment	4		
— mean		66.0	66.7
— range		43—91	47—91
— standard deviation		15.9	18.0
— standard error of mean		7.95	9.00
insulin treatment	4		
— mean		66.0	68.7
— range		49—80	51—80
— standard deviation		13.3	12.9
— standard error of mean		6.65	6.45

Hyaluronic Acid. — The hyaluronic acid concentrations are presented in Table 22. In the hydrocortisone group a slight increase was seen in the means and a decrease in the comparative groups but the changes were statistically not significant.

Intrinsic Viscosity. — In Table 23 are presented the mean values for intrinsic viscosity in the different groups before and

TABLE 22

INFLUENCE OF INTRA-ARTICULAR HYDROCORTISONE TREATMENT ON HYALURONIC ACID CONCENTRATION OF SYNOVIAL FLUID

Group and Characteristic	Number of Sample Pairs	Hyaluronic Acid, g./100 ml.	
		Before Treatment	After Treatment
Hydrocortisone treatment			
poor clinical response	11		
— mean		0.230	0.242
— range		0.15—0.41	0.16—0.37
— standard deviation		0.093	0.078
— standard error of mean		0.0280	0.0235
moderate clinical response	13		
— mean		0.222	0.248
— range		0.13—0.42	0.13—0.51
— standard deviation		0.086	0.114
— standard error of mean		0.0248	0.0316
good clinical response	26		
— mean		0.254	0.270
— range		0.13—0.44	0.14—0.48
— standard deviation		0.092	0.097
— standard error of mean		0.0180	0.0190
total hydrocortisone group	50		
— mean		0.240	0.258
— range		0.13—0.44	0.13—0.51
— standard deviation		0.090	0.097
— standard error of mean		0.0127	0.0137
Comparative groups			
thyroxine treatment	4		
— mean		0.187	0.157
— range		0.15—0.24	0.13—0.17
— standard deviation		0.038	0.019
— standard error of mean		0.0190	0.0095
insulin treatment	4		
— mean		0.190	0.167
— range		0.17—0.20	0.14—0.20
— standard deviation		0.014	0.025
— standard error of mean		0.0070	0.0125

after the hormone treatment. The rise in the viscosity was statistically significant in the groups in which the clinical response to hydrocortisone was clinically poor ($P = 0.05$) or moderate ($P = 0.02$). It was highly significant in the group with a good response ($P = 0.001$) and in the hydrocortisone group as a whole ($P = 0.001$). The changes in the comparative groups were statistically not significant.

TABLE 23

INFLUENCE OF INTRA-ARTICULAR HYDROCORTISONE TREATMENT ON INTRINSIC VISCOSITY OF SYNOVIAL FLUID

Group and Characteristic	Number of Sample Pairs	Intrinsic Viscosity	
		Before Treatment	After Treatment
Hydrocortisone treatment			
poor clinical response	11		
— mean		13.5	15.5*
— range		7.1—19.6	9.8—23.2
— standard deviation		3.78	4.89
— standard error of mean		1.14	1.47
moderate clinical response	13		
— mean		14.5	17.9*
— range		7.3—20.4	10.7—26.6
— standard deviation		4.29	5.17
— standard error of mean		1.19	1.43
good clinical response	26		
— mean		13.9	21.0***
— range		6.5—22.4	10.6—30.2
— standard deviation		4.14	4.45
— standard error of mean		0.81	0.87
total hydrocortisone group	50		
— mean		13.9	19.0***
— range		6.5—22.4	9.8—30.2
— standard deviation		4.04	5.65
— standard error of mean		0.57	0.80
Comparative groups			
thyroxine treatment	4		
— mean		15.2	14.6
— range		11.3—18.0	12.5—20.8
— standard deviation		2.20	4.37
— standard error of mean		1.10	2.18
insulin treatment	4		
— mean		13.3	14.5
— range		9.0—15.8	7.8—19.4
— standard deviation		3.01	5.00
— standard error of mean		1.50	2.50

Colloid Osmotic Pressure. — The changes in the colloid osmotic pressure after different hormone treatments will be seen in Table 24. The decrease in the colloid osmotic pressure was significant in the groups with poor ($P = 0.05$) and moderate ($P = 0.05$) clinical response, and highly significant in the group with good response ($P = 0.001$) and in the entire hydrocortisone group ($P = 0.001$). There was no significant change in the comparative groups.

TABLE 24

INFLUENCE OF INTRA-ARTICULAR HYDROCORTISONE TREATMENT ON COLLOID OSMOTIC PRESSURE OF SYNOVIAL FLUID

Group and Characteristic	Number of Sample Pairs	Colloid Osmotic Pressure, cm. H_2O	
		Before Treatment	After Treatment
Hydrocortisone treatment			
poor clinical response	6		
— mean		42.3	38.2*
— range		37—49	27—44
— standard deviation		4.7	6.2
— standard error of mean		1.92	253
moderate clinical response	7		
— mean		42.3	38.0*
— range		35—48	33—44
— standard deviation		4.2	4.3
— standard error of mean		1.59	1.62
good clinical response	11		
— mean		42.4	33.0***
— range		34—51	25—44
— standard deviation		5.2	5.6
— standard error of mean		1.57	1.69
total hydrocortisone group	24		
— mean		42.4	35.7***
— range		34—51	38—49
— standard deviation		4.6	5.6
— standard error of mean		0.94	1.14
Comparative groups			
thyroxine treatment	4		
— mean		41.7	42.5
— range		39—47	38—49
— standard deviation		5.4	2.2
— standard error of mean		2.70	1.10
insulin treatment	4		
— mean		43.0	44.5
— range		41—46	41—48
— standard deviation		2.2	2.9
— standard error of mean		1.10	1.45

MUTUAL CORRELATIONS OF DIFFERENT PROPERTIES OF SYNOVIAL FLUID

Each of the 28 possibilities of correlation between the sodium, potassium, chloride, protein, protein glucosamine and hyaluronic acid concentrations, intrinsic viscosity, and colloid osmotic pressure of the synovial fluid were studied. The analyses were

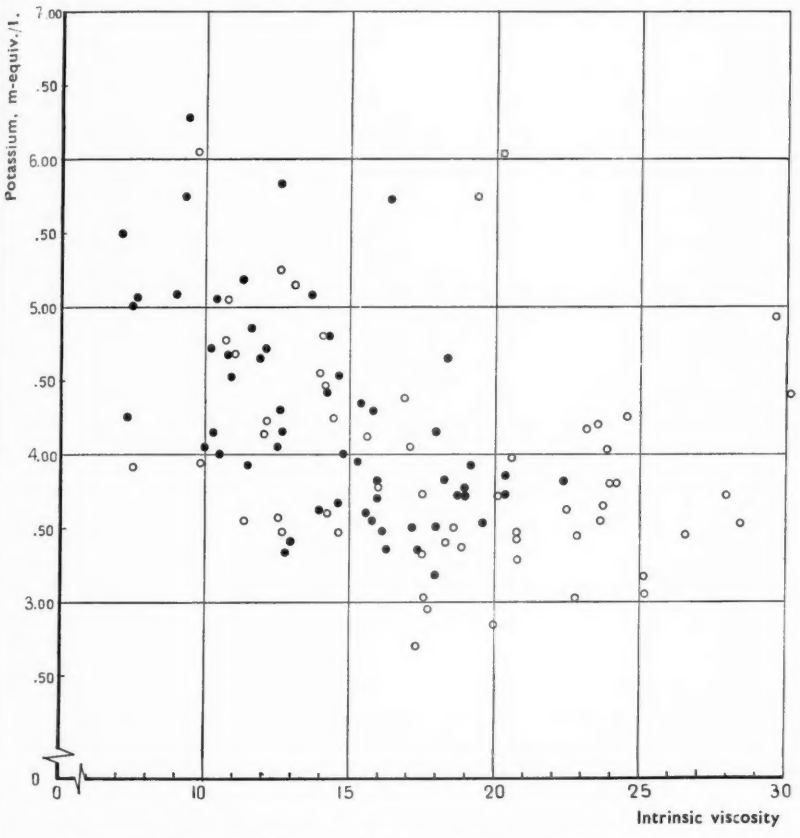

Fig. 5. — Diagram showing the correlation between the potassium concentration and the intrinsic viscosity of synovial fluid

● Before intra-articular hormone treatment ($r = -0.58$ ***)
○ After intra-articular hydrocortisone treatment ($r = -0.26$)

performed separately from the results obtained before hormone treatment and after hydrocortisone treatment.

Electrolytes. — The sodium and chloride concentrations were found to have no relationship with any of the studied chemical or physico-chemical properties of synovial fluid. The negative correlation between the *potassium* concentration and the *intrinsic viscosity* is shown in Fig. 5 (before hormone treatment: $P = 0.001$, after hydrocortisone treatment: $P > 0.1$). Apart from the correlation to the intrinsic viscosity before treatment, the

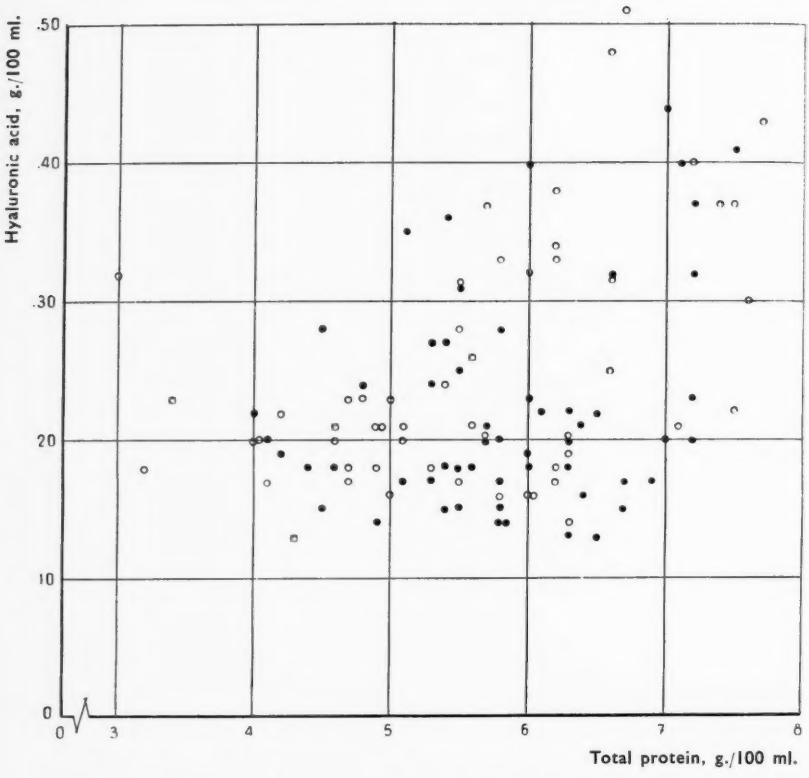

Fig. 6. — Diagram showing the correlation between the total protein concentration and the hyaluronic acid concentration of synovial fluid

○ Before intra-articular hormone treatment ($r = + 0.35$ *)
● After intra-articular hydrocortisone treatment ($r = + 0.57$***)

potassium concentration was not found to be correlated to any of the properties studied.

Total Protein. — No correlation was seen between the total protein concentration and the sodium, potassium, chloride or protein glucosamine concentrations or the intrinsic viscosity. Fig. 6 shows the positive correlation between the total protein and the *hyaluronic acid* concentrations (before hormone treatment: $P = 0.02$, after hydrocortisone treatment: $P = 0.001$) and Fig. 7 the positive correlation between the total protein concentration and the *colloid osmotic pressure* (before hormone treatment: $P=0.001$, after hydrocortisone treatment: $P=0.001$).

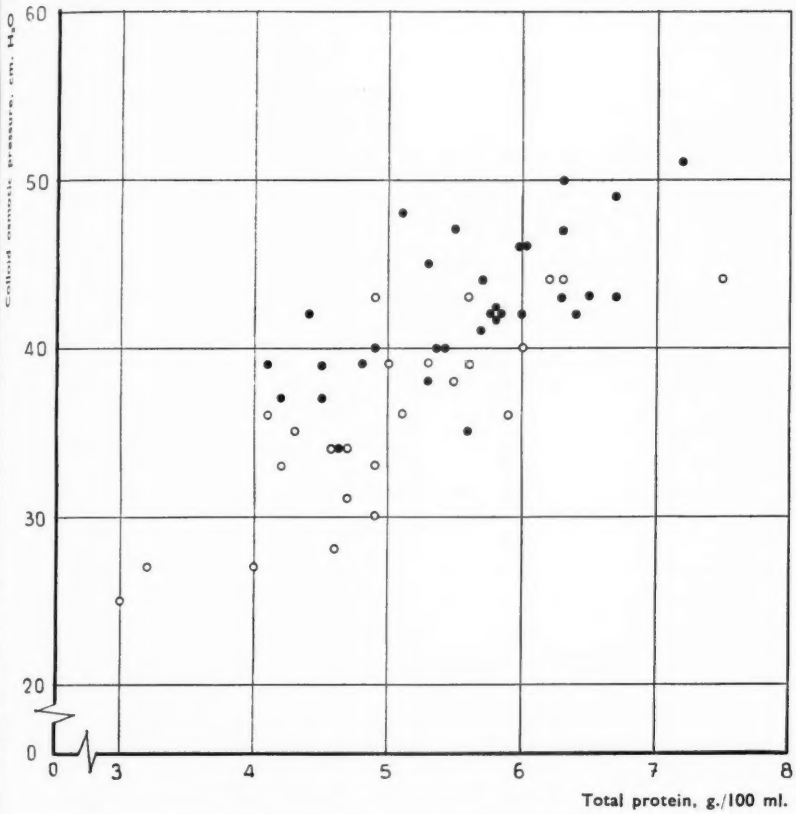

Fig. 7. — Diagram showing the correlation between the total protein concentration and the colloid osmotic pressure of synovial fluid
● Before intra-articular hormone treatment (r = + 0.66 ***)
○ After intra-articular hydrocortisone treatment (r = + 0.82 ***)

Protein Glucosamine. — There was no correlation between the protein glucosamine concentration, on the one hand, and the sodium, potassium, chloride or total protein concentrations, the intrinsic viscosity or the colloid osmotic pressure, on the other hand. The negative correlation to the *hyaluronic acid* concentration is seen in Fig. 8 (before hormone treatment: $P = 0.001$, after hydrocortisone treatment: $P = 0.05$).

Hyaluronic Acid. — No correlation was found to exist between the hyaluronic acid concentration and the sodium, potassium or chloride concentrations. Fig. 6 above showed the positive correla-

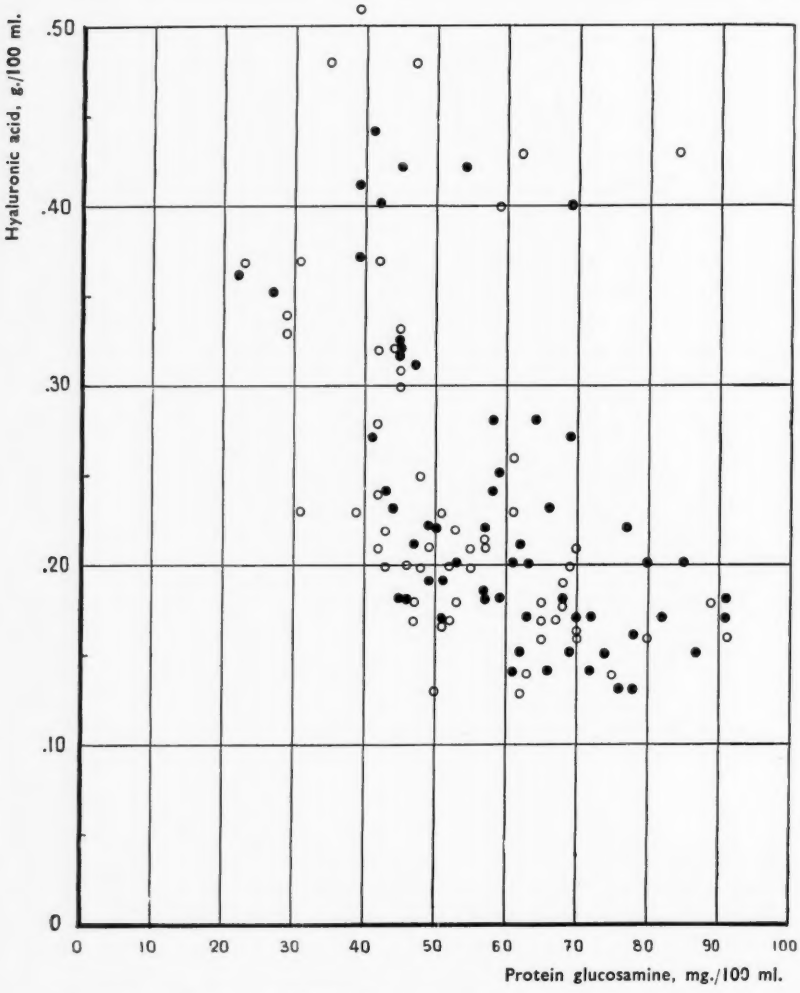

Fig. 8. — Diagram showing the correlation between the protein glucosamine concentration and the hyaluronic acid concentration of synovial fluid

● Before intra-articular hormone treatment ($r = -0.61$ ***)
○ After intra-articular hydrocortisone treatment ($r = -0.35$ *)

tion of the hyaluronic acid concentration to the *total protein* and Fig. 8 the negative correlation to the *protein glucosamine* concentration. Fig. 9 presents the negative correlation to the *intrinsic viscosity* (before hormone treatment: $P = 0.001$, after

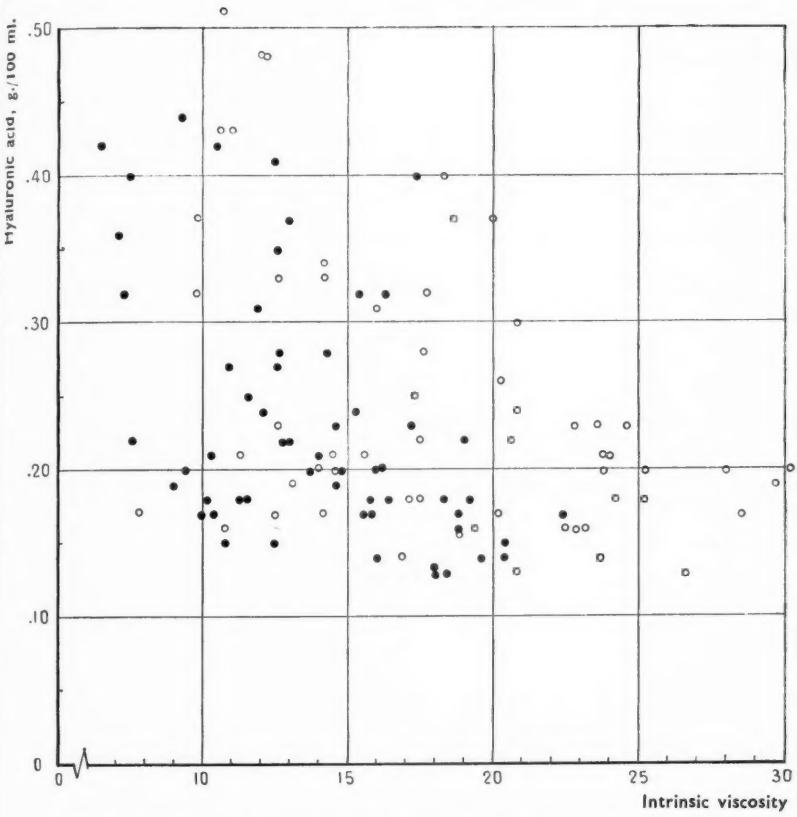

Fig. 9. — Diagram showing the correlation between the hyaluronic acid concentration and the intrinsic viscosity of synovial fluid
● Before intra-articular hormone treatment (r = — 0.51 ***)
○ After intra-articular hydrocortisone treatment (r = — 0.25)

hydrocortisone treatment: $P > 0.1$). There appeared to be no correlation between the hyaluronic acid concentration and the colloid osmotic pressure.

Intrinsic Viscosity. — The negative correlation between the intrinsic viscosity and the *potassium* concentration before hormone treatment was presented in Fig. 5 and the negative correlation between the intrinsic viscosity and the *hyaluronic acid* concentration before hormone treatment in Fig. 9. No correlation was observed between the intrinsic viscosity and the sodium, chloride, total protein or protein glucosamine concentrations.

There also was no correlation between the intrinsic viscosity and the colloid osmotic pressure of the fluid.

Colloid Osmotic Pressure. — The colloid osmotic pressure of the synovial fluid was not related to the sodium, potassium, chloride, protein glucosamine or hyaluronic acid concentrations or to the intrinsic viscosity. The positive correlation to the *total protein* concentration was presented in Fig. 7.

SUMMARY OF STATISTICALLY SIGNIFICANT OBSERVATIONS

The statistically significant observations made concerning the relationship of the chemical and physico-chemical properties of rheumatoid synovial fluid with the clinical activity of the disease, the stage of the disease and the grade of local joint

TABLE 25

STATISTICALLY SIGNIFICANT FINDINGS OF RELATIONS BETWEEN PROPERTIES OF SYNOVIAL FLUID AND CLINICAL FEATURES OF THE DISEASE

Property of Synovial Fluid	Activity of Disease			Stage of Disease			Local Articular Changes		
	Mild or Moderate	Severe	P	I and II	III and IV	P	Mild or Moderate	Severe	P
Potassium concentration, m-equiv./1.	4.02	4.59	0.01	..	..	..	..	..	..
Protein glucosamine concentration, mg./100 ml.	54.0	63.9	0.02	..	..	..	65.1	51.7	0.001
Intrinsic viscosity	15.7	12.3	0.001	13.1	15.5	0.02	..	..	..
Colloid osmotic pressure, cm. H_2O	..	..	..	44.1	39.1	0.001	..	..	..

TABLE 26

STATISTICALLY SIGNIFICANT CORRELATIONS BETWEEN PROPERTIES OF SYNOVIAL FLUID AND THE BLOOD ERYTHROCYTE SEDIMENTATION RATE

Property of Synovial Fluid	*r*	*P*
Potassium concentration, m-equiv./1.	+ 0.41	0.002
Protein glucosamine concentration, mg./100 ml.	+ 0.52	0.001
Intrinsic viscosity	— 0.47	0.001
Colloid osmotic pressure, cm. H_2O	+ 0.70	0.001

TABLE 27

STATISTICALLY SIGNIFICANT FINDINGS OF RELATIONS BETWEEN PROPERTIES OF SYNOVIAL FLUID AND DEGREE OF JOINT EFFUSION IN STAGES I AND II OF THE DISEASE

Property of Synovial Fluid	Degree of Effusion			
	Moderate	Large	Very Large	*P*
Total protein concentration, g./100 ml.	5.71	5.77	6.59	0.01
Colloid osmotic pressure, cm. H_2O	41.8	44.3	46.3	0.05

changes are presented in Table 25. The correlations between the fluid findings and the blood erythrocyte sedimentation rate are shown in Table 26 and the relations of the fluid findings to the degree of joint effusion in Table 27.

The statistically significant changes which occurred in the chemical and physico-chemical properties of the synovial fluid after the intra-articular application of hydrocortisone are presented in Table 28. The changes were more definite in the group of cases in which a good clinical response was obtained to intra-articular hydrocortisone treatment than in those groups of cases in which the response was poor or only moderately good. The intra-articular application of thyroxine or insulin was not found to induce changes that would have been statistically significant.

TABLE 28

STATISTICALLY SIGNIFICANT FINDINGS OF INFLUENCE OF INTRA-ARTICULAR HYDROCORTISONE TREATMENT ON PROPERTIES OF SYNOVIAL FLUID

Property of Synovial Fluid	Before Treatment	After Treatment	*P*
	Mean	Mean	
Potassium concentration, m-equiv./l.	4.23	3.85	0.01
Total protein concentration, g./100 ml.	5.88	5.52	0.02
β-globulin concentration, g./100 ml.	0.73	0.59	0.05
Protein glucosamine concentration, mg./100 ml.	57.9	51.4	0.01
Intrinsic viscosity	13.9	19.0	0.001
Colloid osmotic pressure, cm. H_2O	42.4	35.7	0.001

The statistically significant mutual correlations between the various chemical and physico-chemical properties of the rheumatoid synovial fluid are shown in Table 29.

TABLE 29

STATISTICALLY SIGNIFICANT CORRELATIONS BETWEEN DIFFERENT PROPERTIES OF SYNOVIAL FLUID

Correlation Pairs	Before Intra-Articular Hormone Treatment		After Intra-Articular Hydrocortisone Treatment	
	r	*P*	*r*	*P*
Potassium concentration — intrinsic viscosity	— 0.58	0.001	— 0.26	> 0.1
Hyaluronic acid concentration — total protein concentration	+ 0.35	0.02	+ 0.57	0.001
Hyaluronic acid concentration — protein glucosamine concentration	— 0.61	0.001	— 0.35	0.05
Hyaluronic acid concentration — intrinsic viscosity	— 0.51	0.001	— 0.25	> 0.1
Total protein concentration — colloid osmotic pressure	+ 0.66	0.001	+ 0.82	0.001

DISCUSSION

RELATIONSHIP OF PROPERTIES OF SYNOVIAL FLUID WITH CLINICAL FEATURES OF RHEUMATOID ARTHRITIS

Of the properties of the rheumatoid synovial fluid which were studied in the present investigation, the intrinsic viscosity and the colloid osmotic pressure alone were related to the advancement of the disease. The observation that the intrinsic viscosity was lower in cases in stages I and II than in cases in stages III and IV conforms with the findings reported by Egelius, Jonsson and Sundblad (26). They observed that the anomalous viscosity was lower in cases with slight destructions in the joint than in cases with more marked destructions. The observation made in the present investigation may also be considered to co-ordinate with the finding of Ragan (77) and Ropes and Bauer (89) that the relative viscosity was lower in the early rheumatoid arthritis than in advanced cases.

The differences in means between the groups formed according to the clinical activity of the disease and the correlations of the fluid findings to the blood erythrocyte sedimentation rate point to an increase in the potassium and protein glucosamine concentrations of synovial fluid and to a decrease in the intrinsic viscosity and an elevation of the colloid osmotic pressure with increasing rheumatoid activity. Ropes and Bauer (89) found a higher protein concentration and a lower hyaluronic acid concentration in severe cases as compared with mild cases, but in the present investigation these concentrations were not found to be related to the clinical findings indicating the degree of activity of the disease. Egelius, Jonsson and Sundblad (26), also, were unable to demonstrate any relationship between the hyaluronic acid concentration and the activity of the disease.

However, Sundblad (102) found a direct correlation between the total amount of protein in the fluid and the activity of the disease. The observation made in the present investigation of a relationship between the intrinsic viscosity of the fluid and the clinical activity of the disease is in agreement with the reports of Ragan and Meyer (79), Ropes and Bauer (89) and Sundblad (102).

According to the theory that the synovial fluid is liquid ground substance of connective tissue it is probable that the changes encountered in the synovial fluid, especially in its hyaluronic acid, reflect inflammatory changes produced by the disease in the synovial tissue and possibly even in the connective tissue of the body in general. It has been suggested that changes in the structure and total amount of the hyaluronic acid in the synovial fluid indicate a systemic change in the hyaluronic acid of connective tissue. Egelius, Jonsson and Sundblad (26) were, however, unable to demonstrate any change from the normal in the degree of polymerization of the hyaluronic acid of the synovial fluid aspirated from the unaffected joints of patients with rheumatoid arthritis. They concluded that the hyaluronic acid changes must be due to a local connective tissue disturbance. In the present series a correlation must be regarded to have existed, in most cases, between the severity of the inflammation of the joints under examination and the general activity of the rheumatoid disease. No conclusions concerning the entire connective tissue system can be drawn from the observed relations between the properties of synovial fluid and the clinical observations indicative of the activity of the disease. Basically it may only be a question of a relationship between changes in the synovial fluid and the severity of the local joint inflammation which may parallel the general activity of the disease.

The difference observed between the groups formed according to the grade of the local joint changes with regard to the protein glucosamine concentration of the fluid, and the difference between the groups showing the stage of the disease with regard to the intrinsic viscosity and the colloid osmotic pressure indicate a change towards normal in the synovial fluid with the progression of rheumatoid changes. This observation may probably

be interpreted as a sign of a subsiding trend in the inflammatory process and of reparative phenomena occurring in the synovial tissues with advance of the disease.

EFFECT OF INTRA-ARTICULAR HYDROCORTISONE TREATMENT ON SYNOVIAL FLUID

Yielding, Platt and Holley (111) observed that local treatment with hydrocortisone produced a decrease in the sodium concentration of rheumatoid synovial fluid but they found no change in the potassium concentration. In the present study the findings were in disagreement. The potassium concentration declined, whereas no definite change occurred in the sodium concentration.

The observations reported in the literature concerning the effect of hydrocortisone treatment on the protein concentrations of rheumatoid synovial fluid diverge in many respects. In similarity to the results obtained by Robecchi, Daneo and Riccardino (86), the total protein decreased also in the present study. Other workers (24, 50, 109, 111) have found no change in the total protein concentration. In the albumin concentration Borrachero (14) observed an absolute increase and Robecchi, Daneo and Riccardino (86) a relative increase. In the present work the albumin concentration rather tended to decrease (Table 20, p. 42), but the change was not statistically significant. The observation of a decrease in the β-globulin concentration is in agreement with the report of Borrachero (14). He found also a declined γ-globulin concentration, but in the present study and in that of Robecchi, Daneo and Riccardino (86) such a decrease was not observed.

According to Jessar, Ganzell and Ragan (50) and Sundblad, Egelius and Jonsson (103) the hyaluronic acid concentration of rheumatoid synovial fluid increases after the application of hydrocortisone. No definite effect on the hyaluronic acid concentration was seen in the present investigation, even if there was a slight tendency upwards (Table 22, p. 44). The observed increase in the viscosity in association with hydrocortisone application conforms with the results of earlier investigations (24, 50, 58, 89, 109, 111).

Several of the changes produced by intra-articular application of hydrocortisone in the properties of synovial fluid may be explained in part as a result of resorption *per se*. The observation by Bauer, Short and Bennett (9) concerning the resorption of protein from the joint cavity by way of the lymphatics, and corresponding observations reported in the literature concerning other large-molecular substances and particles (8, 34) are evidently significant from the point of hydrocortisone-induced resorption of a joint effusion.

It has been suggested that the higher degree of polymerization of the hyaluronic acid in the synovial fluid after local hydrocortisone treatment is a result of the influence of the hormone on the synthesis of hyaluronic acid in synovial tissue. However, this opinion is not wholly in agreement with the observations made concerning the inhibitory action of corticosteroids on the hyaluronic acid synthesis (95). The rise in the mean polymerization degree of the hyaluronic acid in the synovial fluid could rather be the result of the elimination of low-polymer hyaluronic acid.

Observations made with regard to the effect of the intra-articular application of hydrocortisone on the properties of the synovial fluid have given evidence of a general normalization of the fluid and point to a partial restoration of normal conditions in the synovial tissue. Among other effects of corticosteroids on the connective tissue are known the inhibitory action on cell proliferation (78, and others) and hyaluronic acid synthesis (95) and the decreasing effect on ground substance (2, 62, 78, and others). It appears probable that actions of this kind contribute to the changes induced by hydrocortisone in the synovial tissue, as a result of which the synovial fluid is partly normalized.

FORMATION OF JOINT EFFUSION

The physiological changes in the volume of synovial fluid are evidently based in the main on the equilibrium between the hydrostatic pressure in the capillaries of the synovial tissue, the colloid osmotic pressure of the plasma, and the colloid osmotic pressure of the synovial fluid. The formation of a joint effusion in pathological conditions probably is — at least for the greater

part — the result of a change in the reciprocal relationship between these three factors.

Theoretically it is possible that the increased colloid osmotic pressure of the synovial fluid may be due to higher protein or hyaluronic acid concentrations or to changes in the physicochemical properties of the hyaluronic acid. In the present study a correlation was seen between the protein concentration and the colloid osmotic pressure of the synovial fluid, but not between the hyaluronic acid concentration and the colloid osmotic pressure or between the intrinsic viscosity and the colloid osmotic pressure. These observations are evidence pointing to the probability that the increased protein concentration of the synovial fluid is the principal reason for the increase in the colloid osmotic pressure. They do not, however, refute a possibility that also the increased amount of hyaluronic acid and the decreased degree of polymerization of the hyaluronic acid may play some rôle in the elevation of the colloid osmotic pressure of the fluid.

Inhibition of the resorption of proteins and hyaluronic acid from the joint cavity is probably one of the reasons for accumulation of these substances in the synovial fluid. Inhibition of the resorption, however, cannot be the explanation for their rapid accumulation in the joint cavity in cases of acute effusion. Probably a more important reason than inhibited resorption for the increase in the total amount of proteins and hyaluronic acid in the synovial fluid is an increased passage of these substances from the synovial tissue into the synovial fluid.

It has been demonstrated that the proteins in the synovial fluid originate, almost if not quite entirely, in the plasma (20, 76, 96). Increased permeability of the capillary walls is a condition for the passage of large-molecular proteins into the synovial tissue. The fact that edema may be present in the joint capsule without a simultaneous formation of effusion in the joint cavity seems to indicate that a readier entrance of proteins into the joint cavity requires not only changes in the capillaries but also a certain degree of change in the ground substance of synovial tissue.

The low concentration of hyaluronic acid in edematous synovial fluid (Table 4, p. 16) indicates that the increased flow of fluid through the synovial tissue does not, without the pre-

sence of pathological changes in this tissue, produce an increase in the total amount of hyaluronic acid in the synovial fluid to the extent that is seen in joint effusions of inflammatory origin. The washing into the joint cavity with plasma of ground substance of pathologically changed connective tissue is not alone a satisfactory explanation for the increased amount of hyaluronic acid in the synovial fluid. One of the reasons, and possibly the primary reason, for this event is most probably an increased production of hyaluronic acid in the synovial tissue.

Although the ground substance of synovial tissue and the synovial fluid are anatomically in direct contact (49, and others), there evidently is a physico-chemical »boundary surface» between them which prevents the passage of colloids from the synovial tissue to the synovial fluid. It is probable that the presence of the »boundary surface» is based on the macromolecular properties of the hyaluronic acid of synovial tissue. The alteration in the permeability of the synovial membrane may be a result of the disturbed synthesis of hyaluronic acid, which disturbance has been suggested as the primary fault in the connective tissue diseases (57, 79).

The formation of an inflammatory joint effusion is a complex event, the details of which are not known. Investigations made so far point to the probability that changes in the capillaries of the synovial tissue and disturbances in the synthesis of hyaluronic acid are basic factors in the mechanism of formation of a rheumatoid joint effusion. These changes in the synovial tissue may lead to an increase of the colloid osmotic pressure of the synovial fluid, which event is probably the principal direct causative agent in the formation of the joint effusion.

SUMMARY

1. In the review of the literature a survey is given of the various theories concerning the origin of synovial fluid, of previous investigations on the biochemical characteristics of normal and certain pathological synovial fluids, and of studies on the effect of corticosteroid hormones on connective tissue and the synovial fluid.

2. In the series of 53 patients with rheumatoid arthritis, chemical and physico-chemical examinations were made of the knee joint effusion fluid drawn immediately before and 24 hours after the intra-articular application of hydrocortisone acetate (55 pairs of samples). For the purpose of comparison, thyroxine was substituted for hydrocortisone in 4 cases and insulin likewise in 4 cases. The sodium, potassium, chloride, total protein, protein glucosamine and hyaluronic acid concentrations, protein fractions, intrinsic viscosity and colloid osmotic pressure of the fluid were determined.

Using statistical methods, an analysis was made of the relationship between the studied characteristics of synovial fluid and the clinical activity of the rheumatoid arthritis (system of Bayles and Hall, blood erythrocyte sedimentation rate), the advancement (stage) of the disease (system of Steinbrocker, Traeger and Batterman), and the grade of articular changes (system of Kellgren and Lawrence) and size of effusion in joints under examination. A statistical analysis was similarly made of the effects of the three hormones on the characteristics of the synovial fluid, as well as of the mutual correlations between the different characteristics of the synovial fluid both before application of hormone and after the hydrocortisone treatment.

3. The mean intrinsic viscosity was lower and the colloid osmotic pressure higher in the fluids obtained from cases in

stages I and II than those from cases in stages III and IV. The mean protein glucosamine concentration of the synovial fluid was higher in the cases with slight or no local joint destructions than in the cases with marked destructions (Table 25, p. 53).

4. In the clinically very active cases of rheumatoid arthritis the mean potassium and protein glucosamine concentrations were higher and the intrinsic viscosity lower than in milder cases (Table 25, p. 53). A statistically significant positive correlation, not applicable to the individual cases, was observed between the potassium concentration, the protein glucosamine concentration and the colloid osmotic pressure, on the one hand, and the blood erythrocyte sedimentation rate, on the other hand. The intrinsic viscosity had a negative correlation to the erythrocyte sedimentation rate (Table 26, p. 53).

5. The mean total protein concentration and the colloid osmotic pressure of the synovial fluid were higher in the joint effusions of the largest size and lowest in the smallest effusions in cases in stages I and II (Table 27, p. 54). This relationship was not observed between the characteristics of the synovial fluid and the size of the effusion in the cases in stages III and IV.

6. The intra-articular application of hydrocortisone reduced the potassium, total protein, β-globulin and protein glucosamine concentrations and the colloid osmotic pressure and increased the intrinsic viscosity of the synovial fluid (Table 28, p. 54). Evidence was found of some rise in the γ-globulin concentration (Table 20, p. 42). Thyroxine or insulin injected into the joint cavity did not produce definite changes in the studied characteristics of the synovial fluid.

7. A statistically significant positive correlation, not applicable to individual cases, was observed between the hyaluronic acid concentration and the total protein concentration and between the total protein concentration and the colloid osmotic pressure. A negative correlation was found between the potassium concentration and the intrinsic viscosity, between the hyaluronic acid concentration and the protein glucosamine concentration, and between the hyaluronic acid concentration and the intrinsic viscosity of the synovial fluid (Table 29, p. 55).

BIBLIOGRAPHY

1. Allison, N., Fremont-Smith, F., Dailey, M. F., and Kennard, M. A.: Comparative Studies between Synovial Fluid and Plasma. — J. Bone & Joint Surg. 1926: *8:* 758.
2. Asboe-Hansen, G.: Effect of the Adrenocorticotropic Hormone of the Pituitary on Mesenchymal Tissue. — Scandinav. Clin. & Lab. Invest. 1950: *2*: 271.
3. Asboe-Hansen, G.: The Variability in the Hyaluronic Acid Content of the Dermal Connective Tissue under the Influence of Thyroid Hormone. — Acta dermat.-venereol. 1950: *30*: 221.
4. Asboe-Hansen, G.: Hormonal Control of Connective Tissue. — Contemporary Rheumatology: Proceedings of the Third European Rheumatology Congress, The Hague — Scheveningen 1955. Ed. J. Goslings and H. van Swaay. — Elsevier Publishing Company, Haarlem 1956.
5. Autio, L.: Studies on the Determination of Colloid Osmotic Pressure. —Ann. med. exper. et biol. Fenniae 1955: *33*: suppl. 5.
6. Autio, L., and Salminen, S.: A New Micromethod for the Determination of Colloid Osmotic Pressure. — Scandinav. J. Clin & Lab. Invest., in press.
7. Bauer, W., Bennett, G. A., Marble, A., and Claflin, D.: Observations on the Normal Synovial Fluid of Cattle. I. The Cellular Constituents and Nitrogen Content. — J. Exper. Med. 1930: *52:* 835.
8. Bauer, W., Ropes, M. W., and Waine, H.: The Physiology of Articular Structures. — Physiol. Rev. 1940: *20:* 272.
9. Bauer, W., Short, C. L., and Bennett, G. A.: The Manner of Removal of Proteins from Normal Joints. — J. Exper. Med. 1933: *57:* 419.
10. Bayles, T. B., and Hall, M. G.: Yardstick for Rheumatoid Arthritis Applied to Patients Receiving Gold Salts. — New England J. Med. 1943: *228:* 418.
11. Bichat, X.: Traité des membranes en général et des diverses membranes en particulier. — Brosson, Gabon, Cie, Paris 1799. Quoted by Kling, (56).
12. Blix, G.: The Determination of Hexosamines According to Elson and Morgan. — Acta chem. scandinav. 1948: *2:* 467.

13. Bollet, A. J.: The Intrinsic Viscosity of Synovial Fluid Hyaluronic Acid. — J. Lab. & Clin. Med. 1956: *48:* 721.

14. Borrachero, J.: Hidrocortisona intraarticular en las artritis y artrosis (Con estudios sobre liquido sinovial). — Rev. clín. españ. 1956: *62:* 235.

15. Cajori, F. A., Crouter, C. Y., and Pemberton, R.: The Physiology of Synovial Fluid. — Arch. Int. Med. 1926: *37:* 92.

16. Cajori, F. A., and Pemberton, R.: The Chemical Composition of Synovial Fluid in Cases of Joint Effusion. — J. Biol. Chem. 1928: *76:* 471.

17. Castor, C. W., and Baker, B. L.: The Local Action of Adrenocortical Steroids on Epidermis and Connective Tissue of the Skin. — Endocrinology 1950: *47:* 234.

18. Coggeshall, H. C.: Comroe's Arthritis and Allied Conditions. 5th ed., ed. J. L. Hollander. — Henry Kimpton, London 1953.

19. Collins, D. H.: The Pathology of Synovial Effusions. — J. Path. & Bact. 1936: *42:* 113.

20. Curtain, C. C.: The Nature of the Protein in the Hyaluronic Complex of Bovine Synovial Fluid. — Biochem. J. 1955: *61:* 688.

21. Davies, D. V.: Anatomy and Physiology of Diarthrodial Joints. — Ann. Rheumat. Dis. 1945: *5:* 29:

22. Davies, D. V.: Synovial Membrane and Synovial Fluid of Joints. — Lancet. 1946: *2:* 815.

23. Dorfman, A.: Connective Tissue in Health and Disease, Ed. G. Asboe-Hansen. — Ejnar Munksgaard, Copenhagen 1954.

24. Duff, I. F., Robinson, W. D., and Smith, E. M.: Evidence of Direct Action of Adrenocortical Steroids on Connective Tissue: The Effects of Systemic Administration of ACTH and Cortisone, and of Intra-Articular Cortisone and Compound F on the Joint Fluid in Rheumatoid Arthritis. — J. Lab. & Clin. Med. 1951: *38:* 805.

25. Edlund, T., Juhlin, L., and Palis, A.: Studies on the Permeability of Connective Tissue. III. The Effects of DOC and Cortisone on the Resistance to Flow of Synovial Membranes. — Acta pharmacol. et toxicol. 1955: *11:* 111.

26. Egelius, N., Jonsson, E., and Sundblad, L.: Studies of Hyaluronic Acid in Rheumatoid Arthritis. — Ann. Rheumat. Dis. 1956: *15:* 357.

27. Ekman, B., Thune, S., and Truedsson, E.: Investigation of Viscosity and Hyaluronidase Inhibitors of Joint Exudate after Intra-Articular Cortisone Injection. — Scandinav. J. Clin. & Lab. Invest. 1953: *5:* 175.

28. Eränkö, O., and Kihlberg, J.: Quantitative Methods in Histology and Microscopic Histochemistry. — S. Karger, Basel 1955.

29. Fischer, A.: Beiträge zur Kenntnis der Gelenkergüsse bei Infektarthritis. — Klin. Wchnschr. 1931: *10:* 1385.

30. Fisher, R. A.: Statistical Methods for Research Workers. 11th ed., revised. — Oliver & Boyd, Edinburgh and London 1950.
31. Fremont-Smith, F., and Dailey, M. E.: Studies on the Distribution of Chloride and Protein between Plasma and Synovial Fluid. — J. Biol. Chem. 1926: *70:* 779.
32. Frerichs, F. T.: Handwörterbuch der Physiologie. Ed. R. Wagner. — Braunschweig 1846. Quoted by Bauer et al., (8).
33. Freyberg, R. H.: Comroe's Arthritis and Allied Conditions. 5th ed., ed. by J. L. Hollander. — Henry Kimpton, London 1953.
34. Gardner, E.: Physiology of Movable Joints. — Physiol. Rev. 1950: *30:* 127.
35. Giansiracusa, J. E., Ropes, M. W., Kulka, J. P., and Bauer, W.: The Natural Course of Rheumatoid Arthritis and the Changes Induced by ACTH. — Am. J. Med. 1951: *10:* 419.
36. Grassmann, W., and Hannig, K.: Ein einfaches Verfahren zur kontinuierlichen Trennung von Stoffgemischen auf Filterpapier durch Electrophorese. — Naturwissenschaften, 1950: *37:* 397.
37. Green, H. N.: Suggested Mode of Action of Corticotrophin in Rheumatoid Arthritis and the Allergic State. — Brit. M. J. 1950: *1:* 1165.
38. Hagen-Torn, O.: Entwickelung und Bau der Synovialmembranen. — Arch. mikr. Anat. 1882: *21:* 591.
39. Hald, A.: Statistical Tables and Formulas. — John Wiley & Sons, Inc., New York 1952.
40. Hammar, J. A.: Ueber den feineren Bau der Gelenke. Abth. I: Die Gelenkmembran. — Arch. mikr. Anat. 1894: *43:* 266.
41. Havers, C.: Osteologia nova. — S. Smith, London 1691. Quoted by Soubbotine (100).
42. Hench, P. S., Kendall, E. C., Slocumb, C. H., and Polley, H. F.: Effects of Cortisone Acetate and Pituitary ACTH on Rheumatoid Arthritis, Rheumatic Fever and Certain Other Conditions: a Study in Clinical Physiology. — Arch. Int. Med. 1950: *85:* 545.
43. Hirsch, C.: Some Views on the Pathology of Synovial Fluid. — Acta orthop. scandinav. 1950: *20:* 121.
44. Hollander, J. L.: Quoted by R. H. Freyberg (33).
45. Hollander, J. L., Brown, E. M., Jr., Jessar, R. A., and Brown, C. Y.: Hydrocortisone and Cortisone Injected into Arthritic Joints: Comparative Effects of and Use of Hydrocortisone as Local Anti-arthritic Agent. — J. A. M. A. 1951: *147:* 1629.
46. Holm-Jensen, I.: A Method for Rapid Determination of Colloid Osmotic Pressure. — Scandinav. Clin. and Lab. Invest. 1949: *1:* 70.
47. Horiye, K.: Über die menschliche Synovia. — Virchow's Arch. path. Anat. 1924: *251:* 649.
48. Hunziker, H.: Das Gelenkpunktat bei der primär-chronischen Polyarthritis. — Ztschr. Rheumaforsch. 1955: *14:* 217.

49. Hüter, C.: Zur Histologie der Gelenkflächen und Gelenkkapseln, mit einem kritischen Vorwort über die Versilberungsmethode. — Virchow's Arch. path. Anat. 1866: *36:* 25.

50. Jessar, R. A., Ganzell, M. A., and Ragan, C.: The Action of Hydrocortisone in Synovial Inflammation. — J. Clin. Invest. 1953: *32:* 480.

51. Keefer, C. S., Myers, W. K., and Holmes, W. F., Jr.: Characteristics of the Synovial Fluid in Various Types of Arthritis. — Arch. Int. Med. 1934: *54:* 872.

52. Kellgren, J. H., and Lawrence, J. S.: Radiological Assessments of Rheumatoid Arthritis. — Ann. Rheumat. Dis. 1957: *16:* 485.

53. Key, J. A.: Cytology of the Synovial Fluid of Normal Joints. — Anat. Rec. 1928: *40:* 193.

54. King, E. S. J.: The Golgi Apparatus of Synovial Cells under Normal and Pathological Conditions and with Reference to the Formation of Synovial Fluid. — J. Path & Bact. 1935: *41:* 117.

55. Kling, D. H.: The Nature and Origin of Synovial Fluid. — Arch. Surg. 1931: *23:* 543.

56. Kling, D. H.: The Synovial Membrane and the Synovial Fluid (with Special Reference to Arthritis and Injuries of the Joints). — Medical Press, Los Angeles 1938.

57. Kulonen, E.: Studies on Hyaluronic Acid and Hyaluronidase in the Animal Organism. — Acta physiol. scandinav. 1951: *24:* suppl. 88.

58. Kulonen, E., and Mäkinen, P.: An Orientating Study on the Synovial Fluid, with Reference to the Effect of Compound F. — Acta rheumat. scandinav. 1955: *1:* 88.

59. Kulonen, E., and Telkkä, A.: Effect of Adrenal Steroids on Synovial Fluid in Vitro. — Ann. med. exper. et biol. fenniae, 1954: *32:* 347.

60. von Kölliker, A.: Über den Bau der Synovialhäute. — Mitt. Naturforsch. Gesellsch., Zürich, Heft 1849: *1:* 93.

62. Lattes, R., Blunt, J. W., Rose, H. M., Jessar, R. A., de Guise Vaillancourt, and Ragan, C.: Lack of Cortisone Effect in Early Stages of Inflammation and Repair. — Am. J. Path. 1953: *29:* 1.

63. Layton, L. L.: Dermal Speading of Hyaluronidase as Influenced by Prolonged Local Treatment with Certain Steroid Hormones. — Proc. Soc. Exper. Biol. & Med. 1951: *16:* 596.

64. Leppänen, V., Krusius, F-E., and Miettinen, T.: Ein einfacher und genauer Flammenfotometer zur Bestimmung der Blutalkalimetalle. — Ann. med. exper. et biol. Fenniae 1952*: 30:* 305.

65. Levine, M. G., and Kling, D. W.: Rheologic Studies on Synovial Fluid. — J. Clin. Invest. 1956: *31:* 1419.

66. Ogston, A.: On Articular Cartilage. — J. Anat. & Physiol. 1875: *10:* 49.

67. Ogston, A. G., and Stanier, J. E.: On the State of Hyaluronic Acid in Synovial Fluid. — Biochem. J. 1950: *46:* 364.

68. Ogston, A. G., and Stanier, J. E.: The Dimensions of the Particle of the Hyaluronic Acid Complex in Synovial Fluid. — Ibid. 1951: *49:* 585.

69. Ogston, A. G., and Stanier, J. E.: Further Observations on the Preparation and Composition of the Hyaluronic Acid Complex of the Ox Synovial Fluid. — Ibid. 1952: *52:* 149.

70. Ogston, A. G., and Stanier, J. E.: The Physiological Function of Hyaluronic Acid in Synovial Fluid; Viscous, Elastic and Lubricant Properties. — J. Physiol. 1953: *119:* 244.

71. Ogston, A. G., Stanier, J. E., Toms, B. A., and Strawbridge, D. J.: Elastic Properties of Ox Synovial Fluid. — Nature, Lond. 1950: *165:* 571.

72. Olhagen, B.: The Protein Pattern of Joint Exudates. — Acta orthop. scandinav. 1950: *20:* 114.

73. Perlmann, G. E., and Kaufman, D.: Electrophoretic Distribution of Proteins in Serum, Plasma and Synovial Fluids of Patients with Rheumatoid Arthritis. — J. Clin. Invest. 1946: *25:* 931.

74. Perlmann, G. E., Ropes, M. W., Kaufman, D., and Bauer, W.: The Electrophoretic Patterns in Synovial Fluid and Serum in Rheumatoid Arthritis. — Ibid. 1954: *33:* 319.

75. Platt, D., Holley, W. L., and Pigman, W.: Changes in the Electrophoretic Behavior of Arthritic Synovial Fluid Components Following Intra-Articular Steroid Therapy. — J. Lab. & Clin. Med. 1957: *49:* 762.

76. Platt, D., Pigman, W., Holley, H. L., and Patton, F. M.: An Electrophoretic Study of Normal and Post-Mortem Human and Bovine Synovial Fluids. — Arch. Biochem. 1956: *64:* 162.

77. Ragan, C.: Viscosity of Normal Human Synovial Fluid. — Proc. Soc. Exper. Biol. & Med. 1946: *63:* 572.

78. Ragan, C., Howes, E. L., Plotz, C. M., Meyer, K., and Blunt, J. W.: Effect of Cortisone on Production of Granulation Tissue in the Rabbit. — Ibid. 1949: *72:* 718.

79. Ragan, C., and Meyer, K.: The Hyaluronic Acid of Synovial Fluid in Rheumatoid Arthritis. — J. Clin. Invest. 1949: *28:* 56.

80. Reed, C. I., Joseph, N. N., Steck, I. E., and Montgomery, M. M.: Effects of ACTH on Synovial Membrane Potentials in Rheumatoid Arthritis. — Fed. Proc. 1950: *9:* 104.

81. Renkonen (Streng), H. O.: Das Serum und die Gelenkflüssigkeit betreffende Untersuchungen bei Kniegelenkerkrankungen. — Acta Soc. med. fenn. Duodecim, Ser A 1939: *21:* suppl. 2.

82. Retterer, E.: Morphologique et histologique des bourses muqueuses et des cavités péri-tendineuses. — J. de l'anat. et de la physiol. 1896: *32:* 256.

83. Retterer, E.: Ébauche squelettogéne des membres et développement des articulations. — Ibid. 1902: *38:* 580.
84. Risak, E., and Winkler, W.: Zur Klinik und Pathologie der Gelenkergüsse. — Deutsch. Arch. klin. Med. 1929: *165:* 129.
85. Robb-Smith, A. H. T.: Connective Tissue in Health and Disease. Ed. G. Asboe-Hansen. — Ejnar Munksgaard, Copenhagen 1954.
86. Robecchi, A., Daneo, V., and Riccardino, N.: Il quadro elettroforetico delle proteine del liquido sinoviale in rapporto al quadro serico e sue variazzioni dopo trattamento con hydrocortone e butazolidina. — Reumatismo, 1955: *7:* 209.
87. Roberts, E., Karnofsky, D. A., and Frankel, S.: Influence of Cortisone on Free Hydroxyproline in the Developing Chick Embryo. — Proc. Soc. Exper. Biol. & Med. 1951: *76:* 289.
88. Robinson, W. D., Duff, I. F., and Smith, E. M.: Joint Fluid Changes in Rheumatoid Arthritis. — J. Michigan M. Soc. 1954: *54:* 270.
89. Ropes, M. W., and Bauer, W.: Synovial Fluid Changes in Joint Disease. — Harward University Press, Cambridge, Mass., 1953.
90. Ropes, M. W., Bennett, G. A., and Bauer, W.: The Origin and Nature of Normal Synovial Fluid. — J. Clin. Invest. 1939: *18:* 351.
91. Ropes, M. W., Robertson, W. V. B., Rossmeisl, E. C., Peabody, R. B., and Bauer, W.: Synovial Fluid Mucin. — Acta med. scandinav. suppl. 1947: *196:* 700.
92. Ropes, M. W., Rossmeisl, E. C., and Bauer, W.: The Origin and Nature of Normal Synovial Fluid. — J. Clin. Invest. 1940: *19:* 795.
93. Schales, O., and Schales, S. S.: Simple and Accurate Method for Determination of Chloride in Biological Fluids. — J. Biol. Chem. 1941: *140:* 879.
94. Schiller, S., and Dorfman, A.: The Metabolism of Mucopolysaccharides in Animals: the Effect of Cortisone and Hydrocortisone on Rat Skin. — Endocrinology 1957: *60:* 376.
95. Schiller, S., and Dorfman, A.: The Metabolism of Mucopolysaccharides in Animals: IV. The Influence of Insulin. — J. Biol. Chem. 1957: *227:* 625.
96. Schmid, K., and Macnair, M. B.: Characterization of the Protein of Human Synovial Fluid in Certain Disease States. — J. Clin. Invest. 1956: *35:* 814.
97. Schneider, J.: Untersuchungen über die Viskosität menschlicher Synovia. — Biochem. Ztschr. 1925: *160:* 325.
98. Schürch, O., Viollier, G., and Süllmann, H.: Elektrophoretische Untersuchungen von Gelenkergüssen. — Schweiz. med. Wchnschr. 1950: *80:* 711.
99. Seifter, J., Baeder, D. H., and Begany, A. J.: Influence of Hyaluronidase and Steroids on Permeability of Synovial Membrane. — Proc. Soc. Exper. Biol. & Med. 1949: *72:* 277.
100. Soubbotine, M.: Recherches histologiques sur la structure des membranes synoviales. — Arch. physiol. norm. et path. 1880: *7:* 532.

101. Steinbrocker, O., Traeger, C. H., and Batterman, R. C.: Therapeutic Criteria in Rheumatoid Arthritis. — J. A. M. A. 1949: *140:* 659.

102. Sundblad, L.: Studies on Hyaluronic Acid in Synovial Fluids. — Acta soc. med. uppsal. 1953: *58:* 113.

103. Sundblad, L., Egelius, N., and Jonsson, E.: Action of Hydrocortisone on the Hyaluronic Acid of Joint Fluids in Rheumatoid Arthritis. — Scandinav. J. Clin. & Lab. Invest. 1954: *6:* 295.

104. Thorn, G. W.: Quoted by R. H. Freyberg (33).

105. Tillmanns, H.: Beiträge zur Histologie der Gelenke. — Arch. mikr. Anat. 1874: *10:* 401.

106. Vaubel, E.: The Form and Function of Synovial Cells in Tissue Cultures, I. Morphology of the Cells under Varying Conditions. — J. Exper. Med. 1933: *58:* 63.

107. Vaubel, E.: Über das Synovialgewebe. — Virchows Arch. path. Anat. 1933: *289:* 670.

108. Wolcott, W. E.: Regeneration of Synovial Membrane Following Typical Synovectomy. — J. Bone & Joint Surg. 1927: *9:* 67.

109. Ziff, M., Scull, E., Ford, D., McEwen, C., and Bunim, J. J.: Effects of Intra-Articular Hydrocortisone Acetate on the Clinical Course, Aminotripeptidase Activity and Other Changes in the Synovial Fluid in Rheumatoid Arthritis. — Ann. Rheumat. Dis. 1952: *11:* 301.

110. Yielding, K. L., Platt, D., and Holley, A. L.: Synovial Fluid, I: Comparison of Sodium and Potassium Concentrations in Normal and Diseased Joint Fluid. — Proc. Soc. Exper. Biol. & Med. 1954: *85:* 665.

111. Yielding, K. L., Platt, D., and Holley, H. L.: Some Effects of Intra-Articular Injection of Hydrocortisone Acetate on Synovial Fluid in Rheumatoid Arthritis. — J. Lab. & Clin. Med. 1956: *48:* 201.

ERRATA

Page 25, line 15:	has	should be	had
» 27, » 21:	Miettinen	— » —	Mettinen
» 28, » 28:	capill-ary	— » —	capil-lary
» 57, » 10:	hya-luronic	— » —	hyal uronic
» 63, » 29:	applic-able	— » —	appli-cable
» 67, » 40:	Miettinen	— » —	Mettinen
» 69, » 20:	Robertson, W. V. B.	— » —	Robertson, W. v. B.